Correlates of Protection

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The determination of a correlate of protection (CoP) by a vaccine is often critical to its development, as its measurement permits a number of both theoretical and practical extrapola- tions. Aside from the intellectual interest in identifying the immune response that is protective, knowledge of a CoP permits measurement of consistent lot potency of a vaccine and differentiation of susceptible from immune individuals. If an infection is uncommon or deadly, such that an efficacy trial is not feasible or ethical, a CoP may yet enable licensure of a vaccine, and once established, a CoP will allow bridging from one vaccine preparation to another.

The terminology in this field is confusing, as various defini- tions have been published, but semantics are important and confusion will be avoided if the definitions used are under- stood. [Table 3.1](#_bookmark0) gives the terminology used in this chapter, and is crucial to its understanding. Whereas a CoP is simply a variable immune response that is statistically associated with protection, the important distinction is between a mechanistic correlate of protection (mCoP) that is directly responsible for protection and a nonmechanistic correlate of protection (nCoP) that is easy to measure but which may only be a sub- stitute for an mCoP that is unknown or difficult to measure. In addition, a CoP may be absolute, in the sense that there is a threshold value above which protection is certain, or relative in the sense that higher values are quantitatively more protec- tive than lower values but there are occasional failures even at high levels and occasional protection at lower levels. Last, and importantly, there are cocorrelates, meaning that more than one immune function induced by a vaccine correlates with protection in an additive or synergistic way. The reader who wishes to learn more on this subject is referred to previously published articles.1–6

The determination of a CoP is sometimes established early

in vaccine development, which is the ideal circumstance in that it makes later vaccine development easier, but sometimes a Phase III trial analysis is necessary to identify the CoP. In the latter case, immune responses of vaccine failures are compared with a sample of subjects who did not become infected. Ideally, the immune responses would be measured at the time of exposure to the infection, but most often samples are avail- able only immediately after vaccination. Nevertheless, a useful CoP is often extracted from those data. Other ways in which CoP are inferred include definition of protective levels of passive antibody given parenterally or acquired by an infant from its mother through the placenta, observations made on vaccinated immunodeficient or immunosuppressed individu- als exposed to the infection, challenge of vaccinated volun- teers by the agent in question, and rarely by extrapolation from challenge of vaccinated animals. Human challenge models have often given critical information about CoP, and should be employed if the challenge is sufficiently attenuated to be ethically acceptable and if a broad range of immune responses are studied. Examples of human challenge that have yielded important information include influenza, cholera, dengue, and cytomegalovirus.

Chapter 2 of this book describes the immunology sur-

rounding vaccination, but the point to bear in mind regarding CoP is that many different functions may serve as a CoP.

Serum and mucosal antibodies come in different isotypes and functionality, and T cells, whether CD4+ or CD8+, can act in a variety of ways, including direct action on infected cells, help to B or other T cells, secretion of cytokines, and even down- regulation of immune responses by T regulatory cells. This chapter seeks to simplify immunology in order to identify principles of protection, although the reader should keep in mind that the immune system is complex and redundant.3 In addition, the reader is directed to chapters on specific vaccines for more extended analysis of each case.

# PRINCIPLES OF PROTECTION

## Principle 1: Protection Must Be Defined in Relation to Specific Phenomena

Protection against infection may relate to different immune markers than protection against disease. Polio is an example in which paralysis can be prevented by serum antibodies, as the virus must pass from the pharynx or intestines to the central nervous system via blood, whereas infection is pre- vented by antibodies at the mucosal level, either locally pro- duced immunoglobulin (Ig) A antibodies or diffusion of IgG antibodies onto the mucosal surfaces of the nasopharynx and intestine. Another instructive example is pneumococcal infec- tions. Prevention of bacteremia can be mediated by antibodies in the range of 0.20 to 0.35 µg of antibody as measured by enzyme-linked immunosorbent assay (ELISA). However, prevention of pneumonia, otitis media, and nasopharyngeal carriage may require levels 10 times higher.7,8

## Principle 2: The Mechanism of Protection by Vaccination Is Not Necessarily the Same Mechanism as Recovery From Infection

The level of antibodies that protect against measles has been defined. A level of 120 mIU of antibody measured by ELISA indicates protection against clinical measles and infection.9 A level between 200 and 1000 mIU confers protection against measles disease, but not always against infection as indicated by antibody rises; a level of 1000 mIU does provide protection even against subclinical infection. However, vaccinated B-cell– deficient humans do recover from disease, whereas T-cell– deficient humans may suffer continued viremia leading to serious and fatal measles. It has been shown in monkeys that the CD8+ T cells specific for measles are required to suppress viremia.10

The poxviruses provide another example. An individual given vaccinia virus requires both B and T cells to overcome the replicating virus and to become immune. However, in a previously vaccinated person only neutralizing antibodies at levels between 1/20 and 1/32 are required for protection, although CD8+ T-cell responses are helpful in the absence of antibodies. Inasmuch as antibody titers decline by 20 years postvaccination, the presence of antipoxvirus T cells enables the vaccinee to have a mild secondary infection in case of exposure.11–13

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## Principle 3: A Large Challenge Dose Can Overcome Immunity

Vaccination against poliomyelitis is usually highly effective in the prevention of paralysis, as stated above, because of the induction of serum antibody. However, gut immunity is imperfect and can be overcome by large infectious doses. A study challenged subjects previously given live vaccine and killed vaccine with two different doses of the oral live vaccine: 800 or 600,000 TCID50 (median tissue culture infective dose). The live vaccine recipients were infected by the low-dose challenge 3% of the time and by the high-dose challenge 15% of the time, whereas 30% of the killed vaccine recipients could be infected by a low dose and 70% by a high dose, indicating that high challenge doses can overcome intestinal immunity.14

Another challenge study involved an experimental cyto- megalovirus vaccine. Seronegative, naturally seropositive, and previously vaccinated subjects were challenged parenterally with 10, 100, or 1000 PFU of a low-passage natural strain of cytomegalovirus. Seronegative subjects were infected with the lowest dose, whereas naturally seropositive subjects were resis- tant to 10 or 100 PFU; however, some could be infected by 1000 PFU. The vaccine prevented infection by 100 PFU of the challenge virus, but not by 1000 PFU.15

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| **TABLE 3.1** Definitions of Terms Used in This Chapter | |
| **Term** | **Definition** |
| Correlate of protection (CoP) | An immune response that is statistically correlated with protection. |
| Mechanistic correlate of protection (mCoP) | The immune response that is responsible for protection. |
| Nonmechanistic correlate of protection (nCoP) | An immune response that substitutes for the true immunologic correlate of protection, which may be unknown or not easily measurable. |
| Absolute correlate | A specific level of response highly correlated with protection; a threshold. |

## Principle 4: Most Current Vaccines Protect Through Antibodies

[Table 3.2](#_bookmark1) lists the commonly used licensed vaccines and their predominant mechanisms for causing disease. Many viral and bacterial agents reach target organs through viremia or bacteremia; consequently, it is easy to understand that antibodies can prevent that passage. Some agents replicate only on the mucosa, but there, too, the local presence of antibody is preventive. The pathogenesis of toxin-producing bacteria such as diphtheria and tetanus can be restricted by antitoxic antibodies. In the case of rabies, replication occurs in the subcutaneous tissue before attachment to neurons and elicitation of antibodies before that attachment prevents rabies. Only in the cases of zoster and tuberculosis vaccines does it appear that antibodies are not the primary mecha- nism of control, but that stimulation of specific T-cell subsets correlate with protection.16 Aside from these theoret- ical arguments, the fact is that passive administration of antibody works for many infections also preventable by vac- cination. Indeed, the efficacy of hepatitis A vaccine was pre- dictable from the fact that induced antibody levels are a thousand times higher than those shown to protect after administration of gamma globulin.17 As mentioned under Principle 1, higher levels of antibody are necessary to prevent mucosal colonization than disease, as for example, in the case of pharyngeal colonization after *Haemophilus influenzae* type b vaccine.18

## Principle 5: Correlates May Be Relative

Although high levels of antibodies are more protective than lower levels, after some vaccines breakthroughs may occur at high levels, even if less frequently than at lower levels. [Fig. 3.1](#_bookmark2) shows a retrospective analysis of the relationship between antibody responses to influenza hemagglutinin and protec- tion against disease. At an anti-HA (hemagglutinin) titer of 1/40, generally thought to be an acceptable response, only approximately 50% of vaccine recipients are protected; even at titers four times higher, breakthrough infections occur.19 Similarly, high levels of pertussis antitoxin after immunization was related to protection both in household exposures and nonhousehold exposures, but mild disease occurred at inter- mediate levels of antibody. Owing to greater exposure, more

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| **TABLE 3.2** Major Licensed Viral and Bacterial Vaccines for Humans According to the Mechanism of Disease Prevented by the Vaccine | |
| **Viral** |  |
| Viremia: | Smallpox, yellow fever, measles Mumps, rubella, polio, varicella Hepatitis A, hepatitis B  Japanese encephalitis, tickborne encephalitis |
| Mucosal replication: | Influenza, rotavirus, human papillomavirus |
| Neuronal invasion: | Rabies |
| Neuronal reactivation: | Zoster |
| **Bacterial** |  |
| Bacteremia: | *Haemophilus influenzae* type b, Meningococcal, Pneumococcal, Typhoid (Vi) |
| Mucosal replication: | Pertussis, typhoid (Ty 21a) |
| Toxin production: | Diphtheria, tetanus, pertussis Cholera, anthrax |
| Macrophage replication: | Tuberculosis |
| *From Plotkin S. Vaccination against the major infectious diseases.* C R Acad Sci III. *1999;322(11):943–951.* | |

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**Figure 3.1.** Protection against influenza and anti-HA (hemagglu- tinatinin) antibodies. *(L. Coudeville, personal communication.)*



100%

90%

80%

70%

60%

50%

40%

30%

20%

10%

0%

0

50 100 150 200 250 300

Influenza antibody



200

NEUT. antibodies

100

0

Natural infection

HPV77DE5

RA27/3

100

HI antibodies

50

0

5yr. 2 mo. 3 yr. 2 mo. 3 yr.

No. tested 19 75 46

Level of protection

Geometric mean titers

**Figure 3.2.** Antibody response to rubella vaccines. HI, hemag- glutination inhibition; NEUT., neutralization.

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| **TABLE 3.3** Pertussis Toxin Antibody as Correlate of Protection Against Pertussis Disease (Modsan Titers) | | |
| **Symptoms** | **Exposure** | |
| **Household** | **Nonhousehold** |
| Severe | 79 U/mL | 99 U/mL |
| Mild | 156 U/mL | 124 U/mL |
| None | 246 U/mL | 155 U/mL |

antibody was required to prevent infection within households than outside households ([Table 3.3](#_bookmark3)).20

## Principle 6: Antibodies Must Be Functional

Although neutralization of viruses and killing of bacteria are often mCoP, it has been increasingly realized that there are other important antibody functions. First, [Fig. 3.2](#_bookmark4) shows a case in which neutralization was definitely important, where titers were measured by neutralization or by hemagglutination- inhibition after vaccination with two different strains of rubella vaccines. As can be seen, both strains induced hemag- glutination inhibiting antibodies, but RA27/3 stimulated con- siderably more neutralizing antibodies and proved to be the more protective vaccine.21

A study of meningococcal Group C polysaccharide vaccine conducted in Quebec showed the difference in function between antibodies measured by ELISA or by bacterial killing. Adults produced both types of antibodies and were highly protected; children 2 to 5 years old produced more antibodies and had moderate protection; and 1-year-old infants gener- ated ELISA but little bactericidal antibody and had no protection.22

A striking example of the importance of antibody function has been reported after an HIV vaccine trial in Thailand. A regimen of priming with a canarypox vector presenting the viral envelope followed by a boost with envelope protein generated little neutralizing antibody, but did generate signifi- cant levels of antibody-dependent cellular cytotoxic antibody that potentiates natural killer cell activity. IgG3 antibody- dependent cell-mediated cytotoxicity (ADCC) antibodies directed against the V1-V2 loops of virus were shown to cor- relate with protection. This illustrated that there are many mechanisms by which antibody may protect.23–26

## Principle 7: T-Cell Responses May Be Correlates

It has usually been assumed that T-cell responses are the important protective correlate for bacille Calmette-Guérin (BCG) vaccine and tuberculosis in general, but it has been difficult to prove that assumption. Interferon-γ secretion has been thought to be important. A study of *Mycobacterium bovis* showed a correlation between interferon-γ–secreting T cells and protective immunity in cows.27 However, although pre- sumably related to T-cell function, it is uncertain as to which, and the mCoP for tuberculosis vaccine in humans remains elusive.28–30 On the other hand, malaria is an example where T-cell responses appear to be crucial, and although antibodies to circumsporozoite protein (CSP) are also generated, they do not appear to be a CoP.31,32 The RTS,S particle containing CSP generates tumor necrosis factor (TNF)-α producing CD4+ cells that appear to predict protection.33 In contrast, a vaccine based on intravenous injection of irradiated sporozoites depends for efficacy on CSP induction of CD8+ T cells that produce inter- feron in the liver, where natural sporozoites must first replicate to launch infection.34

Although vaccination against chlamydia and tularemia

bacteria is still experimental, there is abundant evidence that T-cell responses will be necessary for efficacy.22,35–37

There is clearly much still to be learned about T-cell responses as correlates because of the numerous functional classes of these cells. Although CD8+ T-cell cytotoxicity is clearly one of the most important protective functions, CD4+ T cells exist in many varieties with different helper functions for B and T cells that influence protection directly or indirectly through cytokine secretion.38

## Principle 8: More Than One Factor May Protect as Cocorrelates

A good example of cocorrelates may be derived from studies of influenza. The live, attenuated intranasal vaccine generates both serum antibody and nasal IgA mucosal antibody.39–41 In

vaccinated children challenged with another dose of the intra- nasal vaccine, virus shedding occurred at a high rate in vac- cinees lacking both responses, at a low rate in vaccinees having both responses, and at an intermediate rate if only one response was present. Thus, the two responses were synergis- tic, or at least additive. In the case of inactivated influenza vaccine, two separate antibody responses seem important: that against the viral hemagglutinin and that against viral neuraminidase.42–44 The former block virus entry and the latter block virus exit from the cell. Antibody responses may be poor in the elderly, but granzyme produced by CD8+ cytotoxic T cells are also protective against symptomatic infection by lim- iting replication.45–48

Pertussis vaccines, whether whole cell or acellular, contain multiple components of the bacteria. Toxins and adherence factors are important in pathogenesis, and studies show that whereas antibodies against any of these reduces risk, antibod- ies against multiple virulence factors reduce risk even more.49–51 T-helper cell (Th) 1 and Th17 responses also are important for duration of protection.52–54

A recent illustration of probable cocorrelates is the chim- panzee adenovirus vectored vaccine against Ebola virus. Whereas monkeys that generate ELISA titers of 2000 or more almost always survive an Ebola challenge, passive antibody did not protect and it appeared that CD8+ T cells are needed for protection, particularly of antibody responses are low.55–58

## Principle 9: Memory May Be a Mechanistic Correlate of Protection

Hepatitis B vaccine is highly immunogenic at birth, but anti- bodies are frequently lost later in life. Nevertheless, vaccinees are usually protected because they have an anamnestic anti- body response when exposed to the virus.59–62 Indeed, memory B cells are demonstrable in hepatitis B vaccinees in the absence of serum antibody.63,64

An interesting discovery was made recently in simian immunodeficiency virus (SIV)–infected rhesus monkeys. A rhesus cytomegalovirus vector containing various genes of SIV was constructed. The vector induced effector CD8+ T cells directed against the SIV proteins and was able to abort early SIV infections in the monkeys.65–67 Thus, effector memory of T or B cells is important in diseases for which dissemination occurs early, such as HIV, *H. influenzae* type b and meningo- coccus, but central memory is important in long incubation period diseases such as hepatitis B that allow for mobilization of an anamnestic response.

## Principle 10: There Are Convenient Nonmechanistic Correlates

Zoster is the result of reactivation of varicella virus from dorsal spinal ganglia. The current vaccine consists of live varicella virus in large quantity or the gE protein of the virus. In both cases the vaccines elicit both antibodies and cellular responses. The best CoP is a CD4+ T-cell lymphocyte proliferation index, but there is also a statistical correlation with antibodies to viral glycoproteins.68,68a,68b This suggests that the cellular immunity is an mCoP, whereas the antibody is an nCoP, which makes sense if the vaccines maintain the viral latency in the dorsal root ganglia, but it is not impossible that the neutralization of virus by antibody in the skin is also important.

Another example is from rotavirus vaccination. IgG anti- bodies can prevent infection when given orally or induced by inactivated rotavirus but the replicating live virus vaccines induce mucosal antibody and are effective.69 However, mea- surement of mucosal responses is difficult, whereas measure- ment of serum IgA antibodies is easy. Thus, in the absence of

a generally agreed mCoP, serum IgA provides a very useful nCoP.70–72

# STATISTICAL ISSUES IN DEVELOPING IMMUNE CORRELATES OF PROTECTION

Developing CoPs involves statistical issues in two main areas:

1. development and selection of immunological assays and associated immune response biomarkers meriting study as correlates in preventive vaccine efficacy trials; and (b) statisti- cal evaluation of the selected biomarkers in efficacy trials for their utility as CoPs. For area (a), statistical power for detecting a CoP is eroded for biomarkers with low signal-to-noise ratios73,74 (e.g., from technical assay measurement error), making the standardized statistical evaluation and optimiza- tion of assays within and prior to efficacy trials fundamental for developing CoPs.25,75–78 Moreover, with systems vaccinol- ogy research measuring high-dimensional data on immune responses to vaccination,79–87 an important area of statistical research is classification of vaccinees into low-dimensional biomarker signature groupings79,88–90 for assessment as CoPs. From now on we focus on area (b).

In the clinical trials statistical literature, a CoP is an immune

response biomarker measured after vaccination that can be used to reliably predict vaccine efficacy (VE) against a clinical end point. Several approaches to defining and statistically evaluating CoPs based on efficacy trials have been pro- posed.4,91–103 For CoP assessment, these approaches have been applied to search for thresholds of the biomarker that dis- criminate vaccinees into disease cases (and hence unprotected) versus undiseased controls (and hence putatively pro- tected),9,104–108 or to study how protection depends on the distribution of response.109–111 However, many of these CoP methods do not define protected biomarker subgroups in terms of a causal effect of vaccination, such that an association of the biomarker with disease may not reflect a correlation with VE but rather with pathogen exposure or intrinsic sus- ceptibility to the disease.4,112–115 Addressing this limitation, the “VE curve” approach directly assesses how VE varies across vaccinated subgroups defined by the level of the bio- marker.4,113–119 With this approach, the most useful CoP has VE of zero for vaccinees with negative/absent immune response and VE near 100% for vaccinees with response above a thresh- old. A varicella zoster vaccine (VZV) efficacy trial120 illustrates this approach, where VE to prevent herpes zoster varied widely with fold rise in VZV antibody titers (*P* < .001), with estimated VE of 90% for vaccinees with a 5.26-fold rise compared to 70% and 0% for vaccinees with a 2.39- and zero-fold rise, respec- tively.6 These results provide a benchmark for high protective efficacy. As noted above, a useful CoP may or may not be a mechanism of protection.6

The major challenge of the VE curve approach is that it

requires estimation of how the risk of the clinical end point for a placebo recipient depends on an unmeasured variable— the immune response to the vaccine that the individual would have had, if, counter to fact, the individual had been randomly assigned to receive the vaccine. Consequently, the VE curve statistical methods incorporate techniques for “filling in” the counterfactual immune responses of placebo recipi- ents.113,114,117,119,121–127 The “baseline immune response predic- tor” technique develops a model from vaccine recipients predicting individual immune response biomarkers from baseline variables, and applies this model to placebo recipi- ents using these baseline variables. The “closeout placebo vac- cination” technique crosses over placebo recipients at the end of study follow-up to receive the vaccine and have the immune response directly measured. Crucially, effective use of these approaches requires storing baseline samples in (almost) all

efficacy trial participants, to make possible measurement of preimmunization predictors of the candidate CoP in most disease cases; unavailability of baseline samples has been one of the greatest limiting factors for CoP assessment in efficacy trials.

We conclude by listing five other statistical issues that are faced in developing CoPs in efficacy trials. First, immune responses are typically only measured in a case-control128–130 or case-cohort131–134 subsample, motivating research into opti- mal sampling design117,135–139 and analysis129,130,140 methods.

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| **TABLE 3.4** Correlates of Protection After Vaccinationa | | | |
| **Vaccine** | **Immune Function** | **Protection Level** | **Type** |
| Adenovirus | Nt Ab | 1/4 | M |
| Anthrax | Toxin Nt Ab, anti-PA IgG | 1/3000, 10 µg/mL | M |
| Cholera | Vibrioicidal Ab | ? | NM |
| Dengue | Nt Ab | Variable | M |
| Diphtheria | Toxin Nt Ab | 0.01–0.1 IU/mL | M |
| Ebola | Ab + CMI | ND | M |
| *H. influenzae* conjugate | ELISA Abd | 0.15 ng/mL | M |
| Hepatitis A | ELISA Abd | 20 mlU/mL | M |
| Hepatitis B | ELISA Abd | 10 mlU/mL | M |
| Human papillomavirus | ELISA Abd | Ab, level ND | M |
| Influenza, inactivated | HI Ab | 1/40 = 50% protection, 1/320 in children | M |
|  | NtAb | 1/40 = 50% protection | M |
| Influenza, live | HI Ab, IgA Ab, CMI | ND | M |
| Japanese encephalitis | Nt Ab | 1/10 | M |
| Lyme | ELISA Ab | 1400 U/mL | M (in tick) |
| Malaria | ELISA Ab | >10 U/mL | M |
|  | CD4+ T-cell number | ? | M |
| Measles | ELISA Abd | ≥120 miU/mL | M |
| Meningococcal | Bactericidal Ab | ≥1/4 | M |
| Mumps | Nt Ab | ND | M |
| Pertussis | Ab to PT, Prn, Fim | ND | M |
|  | Th1 T cells | ND | M |
| Pneumococcal, conjugated | ELISA Ab | 0.20–0.35 µg/mL | M (invasive disease) |
| Pneumococcal, polysaccharide | OPA Ab | ND | M |
| Polio, inactivated | Nt Ab | ≥1/8 | M |
| Polio, live | Nt Ab | ≥1/8 | M |
| Rabies | Nt Ab | ≥0.5 IU | M |
| Rotavirus | Serum secretory IgA | ND | NM |
| Rubella | ELISA Ab | ≥10–15 IU/mL | M |
| Smallpox | Nt Abc | ≥1/20–1/32 | M |
| Tetanus | Toxin Nt Ab | 0.01–0.1 IU/mL | M |
| Tick-borne encephalitis | Nt Ab | ≥1/10 | M |
| Tuberculosis | T-cell responses | ND |  |
| Typhoid | b | ND | M |
| Varicella | GP ELISAc,d | ≥5 U/mL | M |
| Yellow fever | Nt Ab | ≥0.7 LNI | M |
| Zoster | CD4+ T cells | ND | M |
| aAlso see specific chapters for references.  bDepending on the specific vaccine, protection against typhoid is mediated by antibodies to flagella, the Vi capsule, O antigen, as well as cell- mediated immunity.  cCellular responses also important.  dSurrogate for neutralizing antibodies.  Ab, antibodies; CMI, cell-mediated immunity; ELISA, enzyme-linked immunosorbent assay; FIM, fimbrial agglutinogens; GP, glycoprotein; HI, hemagglutination inhibition; Ig, immunoglobulin; LNI, log neutralization index; M, mechanistic; MNt, microneutralization; ND, not defined; NM, nonmechanistic; Nt, neutralization; OPA, opsonophagocytic; Prn, pertactin; PT, pertussis toxin. | | | |

Second, statistical methods are needed to develop cocorre- lates, for example, by estimating VE surfaces across vaccinee subgroups defined by the level of two immune response bio- markers114,119 or estimating VE curves for a univariable com- posite biomarker.126 Moreover, for assessing complex correlates3 based on high-dimensional immune response biomarkers, supervised machine learning methods are needed to estimate the best models of VE.88,89,141,142 Third, two types of CoP analy- ses are defined by measuring the immune response biomarker at a fixed time point shortly after vaccination or at serial time points including just before exposure and infection or disease. Both analyses are important, the former primarily for develop- ing practicable VE-predictive end points and the latter primar- ily for generating clues about mechanistic CoPs. Fourth, a highly predictive CoP for a particular disease end point, vac- cine, and study population may fail for a different end point, vaccine, or study population,143 indicating that metaanalysis of multiple efficacy trials combined with knowledge of mecha- nisms of protection is needed for bridging CoPs to new settings.4,55,94,97,106,115,144,145

Lastly, for genetically diverse pathogens, the whole discus- sion above applies to assessing CoPs against particular pheno- types or genotypes of the pathogen (e.g., through pathogen type-specific VE curves), with common application historically to assess serotype- or biotype-specific antibody titers as CoPs against disease with the same type.146–151 In contemporary effi- cacy trials, pathogen genomes infecting study participants are sequenced, enabling a statistical sieve analysis that assesses how VE varies with sequence characteristics.152 Immune response data can inform the sieve analysis by focusing the analysis on putatively protective epitopes such as highly vaccine-reactive peptides or specific reagents that were identi- fied as CoPs.153,154 Moreover, host characteristics other than immune responses (such as demographics, ecological factors, or genetics such as human leukocyte antigen [HLA] types restricting epitope-specific T-cell responses) may affect VE or type-specific VE, such that the overarching goal of VE curve CoP analysis may be stated as estimation of how VE varies jointly in pathogen sequence characteristics, immune response biomarkers, and any other host characteristics.3,73,87,155

# COMPLEXITY OF CORRELATES

As Einstein said, “Everything must be made as simple as pos- sible, but not simpler.” We have attempted above to reduce this subject to simple principles, but one must recognize that in practice the determination of CoPs is difficult. First, it is necessary to repeat that CoP are not restricted to neutraliz- ing IgG antibody or CD8+ T cell cytotoxicity. Other immuno- logic responses, such as antibodies that stimulate natural killer cells,156 Th17 T cells that prevent mucosal infection,157 and T cells that secrete cytokines are examples of possible CoP.158

The CoP for influenza vaccine appears at first to be straight- forward: hemagglutination-inhibiting antibody or perhaps neutralizing antibody. However, in children it appears that higher levels of antibody are necessary, perhaps because of the absence of prior influenza infections.159 In the elderly the situ- ation is even more complex, owing to immunosenescence, such that the presence of cytotoxic T cells may be necessary to protection against serious disease in the presence of a weak antibody response.46

A CoP for pertussis vaccine has long been controversial, but the use of acellular vaccines with a variable number of

components has been educational. As stated above it appears that high-titered antibodies against pertussis toxin, pertactin, and fimbrial hemagglutinogens each provide some degree of protection and that there is synergy among those antibodies.52 However, a T-cell response is critical to duration of antibody and to prevention of nasopharyngeal carriage, and Th17 cells are also important based on evidence from mouse and baboon models.160,161 Thus, B and T cell responses act together for the best efficacy.162

The importance of CD4+ T cell immunity to protection by the RTS,S malaria vaccine based on CSP was discussed above. However, protection induced by the liver stage multiple epitope-thrombospondin–related adhesion protein (ME- TRAP) antigen is mediated by CD8+ T cells163 and the mero- zoite MSP2 and-3 antigensactbyinductionofopsonophagocytic antibodies.164,165

In line with the doctrine that the immune system is redun- dant, there are multiple situations in which antibody is the first line of defense and therefore an mCoP, but where cellu- lar immunity provides a backup in the event antibodies are low. Such may be the case for Ebola virus and yellow fever virus.56,166 For some other agents the two arms of the immune system are synergistic, as in the case of the RTS,S malaria vaccine that induces both types of response against the cir- cumsporozoite antigen of the parasite.167,168 In other cases, the CoP varies with the type of vaccine. Whereas as described above, the canarypox vector/HIV protein vaccine for HIV depends for its efficacy on an ADCC antibody response,156 a rhesus cytomegalovirus vectored vaccine that protected monkeys against SIV acts through induction of effector T cells.66,67 In the case of human cytomegalovirus antibody to surface proteins protect against acquisition by contact,169 but cellular immunity to internal antigens seems to be key in preventing transplant-associated disease in hematogenous stem cell recipients.170

Also still to be defined are CoP relating to mucosal immune

responses. Mucosal IgA antibody produced by vaccination has been identified to be important to protection against influ- enza39,171 polio,172 and respiratory syncytial virus,173 and probably is important for rotavirus vaccine.70,174 Serum IgG antibody diffusing onto respiratory, gastrointestinal, and genital mucosae undoubtedly also can prevent infection but is poorly characterized.

# CONCLUSIONS

The immune system is redundant, but the mCoP after vac- cination in most cases is antibody that blocks the agent in the blood. Antibodies have varying functions and the mCoP will vary according to the vaccine. Cellular immunity may synergize with antibody or make up for its lack. Effector T-cell memory may be necessary for short incubation period dis- eases, whereas central memory with an anamnestic response is sufficient for long incubation period diseases. For some vaccines we do not know the true mCoP but we can use a nCoP that is not protective but is statistically correlated with protection. In general, there is a spectrum of correlates, ranging from pure antibody to the protective antigen in anthrax vaccine to the resurrection of cellular immunity to varicella virus that results from immunization with zoster vaccine.

[Table 3.4](#_bookmark5) gives our best estimates of CoPs for currently licensed vaccines.

References for this chapter are available at [ExpertConsult.com](http://www.ExpertConsult.com/).

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