Chapter 16
Strategies for the Nonclinical Safety Assessment of Vaccines

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16.1 Introduction

Over the past century, vaccines have made a large impact on public health. Prophylactic vaccines prevent disability and disease, saving millions of dollars in potential health-care spending. Since prophylactic vaccines are administered to healthy individuals, including infants and children, it is important to demonstrate the safety of vaccines preclinically prior to testing the vaccine in clinical studies. A benefit-to-risk profile is considered for each individual vaccine and depends on many factors including preclinical and clinical toxicities that are observed, frequency of administration and intended target population. For prophylactic vaccines, in particular, the concerns about potential risks often outweigh the perception of benefit [1]. Therefore, over the past decade, there has been an increased focus on nonclinical safety assessment of vaccines, including toxicity testing.

Traditional vaccines have focused on prevention of infectious diseases by eliciting humoral immune responses, and are typically composed of whole, inactivated, or attenuated microorganisms (bacteria or viruses) that have lost their disease-producing properties [2]. Next generation vaccines are being designed not only for prevention of infectious diseases but also for treating chronic diseases such as hepatitis C or cancer. Next generation vaccines aim to induce strong humoral and cell-mediated immune responses and include both prophylactic and therapeutic vaccines. Next generation vaccines are often produced synthetically or purified from pathogens, and include antigens (proteins, peptides or carbohydrates) capable of inducing humoral and cellular immune responses. These new epitopes are often weak immunogens; therefore, they need to be presented in multimeric form, conjugated, or formulated with immune potentiators such as adjuvants in order to elicit a stronger immune

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response [2]. Next generation vaccines include virus-like particles (VLPs) that are noninfectious but are immunogenic, or can act as carriers to linked peptide-antigens. Next generation vaccines also include DNA vaccines which transfect cells in order to express the antigen of interest, and are delivered either in a plasmid form or vectored using an intact bacteria or virus.

Safety concerns for traditional and next generation vaccines include the potential to induce local and systemic reactions. Local reactions are often observed with vaccines administered via the intramuscular or subcutaneous routes of administration, and there are concerns about the severity of pain, redness, swelling, in addition to formation of granulomas and abcesses at the injection site, necrosis and regional lymphadenopathy [3]. Vaccines might induce systemic reactions, including nausea, diarrhea and general malaise. Potentially severe responses might include anaphylaxis, pyrogenic fever responses, organ specific toxicity, or immune-mediated toxicities (such as cytokine release, immune activation or suppression, and autoimmune diseases). Other potential concerns include effects on reproduction and development, and carcinogenicity. For live or attenuated pathogen-based vaccines, there is a risk of reversion to virulence in addition to concerns regarding administration of the vaccine to subjects who have an impaired immune system. For next generation vaccines which include adjuvants, there are potential synergies and interactions between the mechanisms of action for vaccine antigens and adjuvants. Adjuvants typically act by enhancing the immune response, and might cause excessive amounts of pro-inflammatory and pyrogenic mediators leading to an exacerbation of both local and systemic effects [3]. Next generation vaccines that are DNA-based or vectored have specific risks of recombination and integration into the host genome. Therefore, the biodistribution, integration, and persistence of the DNA or vector are important evaluations for DNA-based and vectored vaccines.

Prior to starting clinical studies with next generation vaccines, adequate information about the pharmacological and toxicological effects of the vaccine should be available [1]. This includes in vitro and in vivo studies to examine the mechanism of action and potential efficacy of the vaccine, in addition to a thorough evaluation of the safety of the vaccine. This chapter will focus on the nonclinical safety assessment of vaccines, and will include a discussion of the toxicology studies that need to be performed for new vaccines in clinical development and quality control tests that are needed to demonstrate that the vaccine product is safe for use in humans.

16.2 Overview of Toxicology Studies for Vaccines

Nonclinical testing of traditional vaccines was focused mainly on efficacy studies in animals and “safety pass” of vaccine formulations. Over time, the extent of nonclinical safety testing has been greatly increased and a requirement for full toxicology studies of vaccine candidates have been implemented according to current guidelines (Table 16.1). Presently, nonclinical safety studies with vaccine candidates, including the next generation vaccines, are aligned with overall principles of

Table 16.1 Regulatory guidelines

Vaccine type	Regulatory agency	Guideline
All vaccines	World Health Organization (WHO)	WHO guidelines on nonclinical evaluation of vaccines [4]
	European Medicines Agency (EMA)	Note for guidance on preclinical pharmacological and toxicological testing of vaccines [5]
Prophylactic vaccines	Ministry of Health, Labour and Welfare, Japan	Guideline for nonclinical studies of vaccines for preventing infectious diseases [6]
	State Food and Drug Administration, China	Technical guidelines for preclinical research on preventive vaccines [7]
DNA vaccines	Food and Drug Administration (FDA), United States of America	Guidance for industry: considerations for plasmid DNA vaccines for infectious disease indications [8]
	WHO	Guidelines for assuring the quality and nonclinical safety evaluation of DNA vaccines [9]
Viral vector and cell-based vaccines	FDA	Guidance for industry: guidance for human somatic cell therapy and gene therapy [10]
	EMA	Guideline on quality, nonclinical and clinical aspects of live recombinant viral vectored vaccines [11]
		Note for guidance on the quality, preclinical and clinical aspects of gene transfer medicinal products [12]
		Points to consider on the manufacture and quality control of human somatic cell therapy medicinal products [13]
Recombinant protein/peptide vaccines	FDA	Points to consider in the production and testing of new drugs and biologicals produced by recombinant DNA technology [14]
	International Conference on Harmonization (ICH)	Preclinical safety evaluation of biotechnology-derived pharmaceuticals [15]. Note: this guideline is more applicable for biological drugs, not vaccines
Adjuvants	EMA	Guideline on adjuvants in vaccines for human use [16]
Combination vaccines	EMA	Note for guidance on pharmaceutical and biological aspects of combined vaccines [17]

(continued)

Table 16.1 (continued)

Vaccine type	Regulatory agency	Guideline
Vaccines for women of childbearing potential	FDA	Guidance for industry: considerations for developmental toxicity studies for preventative and therapeutic vaccines for infectious disease indications [18]
Therapeutic cancer vaccines	FDA	Draft guidance for industry: clinical considerations for therapeutic cancer vaccines [19]
Cell substrates for viral vaccines	FDA	Characterization and qualification of cell substrates and other biological materials used in the production of viral vaccines for infectious disease indications [20]
	WHO	Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks [21]
	ICH	Viral safety evaluation of biotechnology products derived from cell lines of human or animal origin [22]

Table 16.2 Types of toxicology studies

Study type	Purpose	Comment
Single-dose toxicity	To determine the acute effects after vaccination by examining general parameters (mortality, clinical signs, body weight, food consumption)	These acute evaluations are often incorporated within repeat-dose toxicity studies, and separate single-dose toxicity studies do not need to be performed
Repeat-dose toxicity	To determine the effects of repeated administration of the vaccine in animals	This is typically the pivotal toxicology study that is performed prior to clinical trials
Local tolerance	To determine the potential irritation at the injection site	To reduce animal use, a local tolerance evaluation can be incorporated within the repeat-dose toxicity study
Safety pharmacology	To evaluate the potential for undesirable effects on the cardiovascular, respiratory, and central nervous systems	Separate safety pharmacology studies are generally not performed for vaccines [1], and endpoints are incorporated in the repeat-dose toxicity study instead
Developmental and reproductive toxicity studies	To examine potential effects on fertility, fetal development, and postnatal development of the offspring	Required for vaccines that will be indicated for women of childbearing potential [18]
Biodistribution studies	To examine tissue distribution following administration	Performed for nucleic acid and viral vector-based vaccines

toxicology evaluation, that is, the detection of their potential for local and systemic toxicity. At the same time the guidelines allow for appropriate flexibility in study designs according to the type of the vaccine candidate, the human population to be treated, and the dosing regimen to be applied in the clinical use.

The purpose of the nonclinical toxicology evaluation is to examine the toxicity of all the components present in the vaccine formulation in addition to the toxicity of the induced immune response. Toxicology studies provide information that might help to determine a safe starting dose in the clinical study and identify any potential toxicities or target organs [23]. It should be noted that there are some limitations of safety evaluation in animals, since effects in animals are not always indicative of the effect that might be seen in humans, and rare toxicities that appear in certain subpopulations are only detected in clinical studies. Nevertheless, toxicology studies provide important safety data for vaccine development.

The toxicology program for each vaccine varies depending on the type of vaccine and intended use in humans. An overview of the main toxicology study types is provided in Table 16.2. In general, all vaccines need to be evaluated in a repeat-dose toxicology study prior to the start of Phase 1 clinical studies. Developmental and reproductive toxicology studies are needed for vaccines that will be administered to women of childbearing potential and are performed in parallel with Phase 3 clinical studies [23]. Biodistribution studies are needed for DNA-based and viral-vectored vaccines.

Toxicology studies are performed in animals and need to be performed in compliance with national and international laws for the protection of laboratory animals. Toxicology study protocols are reviewed and approved by the Institutional Animal Care and Use Committee (IACUC). Toxicology studies are usually conducted in compliance with Good Laboratory Practices (GLP) [24]. The vaccine lots used in GLP studies should be from lots that are manufactured with a similar production process, formulation, and release specifications as the lots intended for clinical use. Stability data are needed, supporting the use of the vaccine for the duration of the toxicology study.

16.2.1 Repeat-Dose Toxicity Studies

Repeat-dose toxicity studies are generally needed for all vaccine types. A single species is typically used for the evaluation, which must be shown to be a relevant species based on the immunogenicity or efficacy of the vaccine in the selected species. In many cases, rodents or rabbits are used for the toxicology evaluation. Nonhuman primates are only used if no other relevant species exist. Disease models are typically not used for toxicology studies, but supplementary studies in disease models could be used to address specific toxicology concerns. For example, a transgenic mouse model of Alzheimer's disease, which over-expresses the human β-amyloid protein, could be used to demonstrate that Alzheimer's disease vaccine candidates do not cause meningoencephalitis or microhemorrhage in the brain [25].

In repeat-dose toxicity studies, the same route of administration as the clinical route is used in animals; however, a more intensive dosing regimen is applied in animals when compared with the planned regimen for humans. This "overdosing" approach based on the number of doses administered, i.e., one more dose is administered in animals when compared with the number of doses administered to humans, and greater dosing frequency, i.e., every 2–3 weeks in animals compared to typically every few months in humans, is driven by the intent to maximize potential hazard identification in nonclinical safety studies [1]. In addition, the full-human dose of the vaccine or the maximum amount that can be injected into the selected animal species also results in much higher exposure to the vaccine in animals based on their smaller body weight compared to humans. Importantly, the vaccine formulation used in toxicology studies should be representative of the proposed clinical formulation. Therefore, for adjuvanted vaccines, the vaccine antigen(s) and adjuvant are tested together based on the evidence that immune response to adjuvanted vaccine can only be evaluated within the confines of immunogenicity of the vaccine antigens [26].

Control groups that are included in the repeat-dose toxicity study include adjuvant alone, if applicable, and a saline-treated group. The group size varies depending on the animal species used, but for rodents, 10 per gender per group are usually included for each necropsy. For non-rodents the number per group is typically 3–5 per gender per group for each necropsy. Antemortem parameters evaluated include

daily clinical observations, weekly body weights, food consumption, and physical examinations. An assessment of local reactogenicity is performed after each vaccine dose is administered. Specific safety pharmacology evaluations (e.g., body temperature, electrocardiogram, and central nervous system evaluations) could be incorporated within the repeat-dose toxicity study [27]. For vaccines that are administered intramuscularly, particular attention is focused on redness and swelling at the injection site and impairment of limb use after the injection. Clinical chemistry (urinalyses, hematology, serum biochemistry, coagulation) evaluations are typically performed a few days after the first vaccination and at the scheduled necropsies. Immunogenicity assessments are performed at the end of the study (as described in Sect. 3.1). Ophthalmic examinations are included after the first vaccination and prior to the first necropsy. Necropsies are performed on two occasions: (1) 1–3 days after the last dose is administered and (2) after a treatment-free period of 2–4 weeks (to determine whether any effects detected at the first necropsy have started to recover with time). Postmortem evaluations include gross examination of all major organs, organ weights for selected organs, and histopathology evaluation of a standard list of tissues [4].

Treatment-related effects that are typically observed in repeat-dose studies with vaccines administered parenterally, include inflammation at the injection site, hyperplasia of the draining lymph nodes, increases in spleen weight and clinical chemistry changes that are indicative of an inflammatory response. These are typically not severe and are transient changes, and they are therefore not considered to be an adverse effect.

16.2.2 Considerations for Prophylactic and Therapeutic Vaccines

The general approach to the toxicology evaluation for therapeutic and prophylactic vaccines is very similar; however, there are a few small differences in the repeat-dose toxicity study designs. For example, for a therapeutic vaccine, the interval between dose administrations in animals would follow the clinical study design very closely, including the total number of doses and dosing intervals; whereas for a prophylactic vaccine, the dosing interval could be condensed in the animals (e.g., clinical dosing frequency of once-every-3-months could be condensed to once-every-3-weeks in the toxicology study) and one more dose is administered in animals when compared with the number of doses in the clinical regimen. There is a perception that there is a potential difference in the tolerance for adverse effects for therapeutic vaccines when compared with prophylactic vaccines, since therapeutic vaccines address life-threatening conditions for which there might be no other treatment options. However, the benefit-to-risk ratio needs to be carefully evaluated depending on the target population. It should be noted that certain target populations for therapeutic vaccines might be immunosuppressed due to other concomitant medications. Therefore, caution is needed when evaluating the benefit-to-risk ratio for both therapeutic and prophylactic vaccines.

16.2.3 Considerations for Inclusion of Adjuvants in Vaccine Formulations

Novel adjuvants are being incorporated in next generation vaccine formulations in order to reduce the amount of vaccine antigen and increase both the magnitude and duration of the immune response, thereby reducing the frequency of booster immunizations needed. Adjuvants can be used to modify a desired immune response and activate both the innate and adaptive arms of the immune system [2]. Novel adjuvants that are being tested in clinical trials currently include mineral salts (e.g., aluminum hydroxide), oil emulsions (e.g., MF59), particulate adjuvants (e.g., virosomes and ISCOMS), microbial derivatives (e.g., monophosphorylated lipid A), and endogenous immunomodulators (e.g., human GM-CSF). Some adjuvants that were developed in the past (e.g., Freund's adjuvant) were not found to be acceptable for large scale vaccination campaigns due to safety concerns, which included severe local reactions, acute toxicity, and delayed hypersensitivity. EMA's Committee for Medicinal Products issued a guideline on adjuvants in vaccines for human use, which covers the nonclinical and clinical aspects for consideration [16].

The safety profile of an adjuvant alone would be typically impacted (positively or negatively) by its interactions with vaccine antigen(s) and needs to be evaluated in the context of the full vaccine formulation. A theoretical concern about increased toxicities due to synergy between adjuvant-induced responses and vaccine-induced responses has been raised by regulatory authorities. It is proposed that toxicological characterization of chemical-based adjuvants in a manner similar to all new chemical entities (NCEs) is desired in order to understand their unique toxicity profiles [6, 16]. If the adjuvant is not species-specific, it is tested in two species (one rodent and one non-rodent). If the adjuvant is species-specific, then testing in one species might be justified.

New adjuvants are typically assessed for local tolerance and systemic toxicity in a repeat-dose toxicity study. The repeat-dose toxicity study design could reflect the proposed clinical use of the vaccine, and the number of administrations in animals should be higher than the number planned for humans [16]. In general, dose ranging toxicology studies do not need to be performed on the adjuvant alone. The doses tested would reflect the targeted clinical use, which is typically much lower than the maximum tolerated dose. The purpose of the toxicology studies with adjuvant alone is to establish a margin of safety rather than a maximum tolerated dose. Full necropsy and histopathology are included in the repeat-dose toxicity study. Similar to other NCEs, an assessment of genotoxicity potential of novel chemical adjuvants is also recommended using the standard battery of tests (e.g., potential for gene mutation, chromosome aberrations, and primary DNA damage) [28]. Carcinogenicity studies are not required for adjuvants, since they are only used a few times at low doses. An evaluation of the adjuvant's effect on reproductive toxicity is needed for inclusion of the adjuvant in vaccines that will be administered to women of childbearing potential or during pregnancy.

Although the value of studies with an adjuvant alone for risk assessment of a vaccine as a whole is still being debated, in light of these guidelines, it is anticipated that the rigorous and comprehensive toxicology programs for novel adjuvants alone will be required in the future. Perhaps, a practical approach to compliance with these expectations by developers of vaccines containing novel adjuvants could include a generation of a “Master File” for a given adjuvant. With this approach, nonclinical safety studies with an adjuvant alone would be included in the Master File and potentially repetitive safety studies of that adjuvant alone could be omitted.

16.2.4 Considerations for New Approaches to Administration of Vaccines

Different delivery systems are being used to incorporate immunopotentiators and focus the immune response through a desired path. Several types of delivery devices for vaccines are also being evaluated in order to more efficiently target the vaccine to a specific area in the body and reduce the pain associated with needle-based injections.

Delivery systems include emulsions (e.g., MF59) and microparticles (e.g., liposomes and biodegradable polyesters), which might have immunostimulatory capabilities, by themselves [2]. This type of delivery system encapsulates and protects the antigen from degradation, and acts as a vehicle that mimics the structure of natural lipid bilayer membranes, allowing them to enter into the reticulo-endothelial system by endocytosis. Delivery systems could also stabilize the antigen and result in formulations that are thermostable. Delivery systems that are present in vaccine formulations need to be included in the formulation that is used toxicology study in animals. A group of animals that are dosed with the delivery system (e.g., lipsome or emulsion), by itself, could be included in the toxicology studies to compare the effects of the delivery system by itself or in combination with vaccine antigens.

Delivery devices are used to target the vaccine antigens to the proper location in the body and include less painful ways to deliver vaccine antigens parenterally, such as microneedle patches and autoinjectors [29]. Oral and intranasal vaccines are also being developed, in order to have a less invasive method of administering vaccines. Toxicology studies of vaccines that are intended for delivery in the clinic using a specific device should include the use of the clinical delivery device in the animal study [23]. This is particularly important for new types of injection devices, since local irritation is a concern. If the device has already been cleared for use in humans, then a cross-reference to the Investigational Device Exemption or Master File could be listed in the Investigational New Drug application for the vaccine. The manufacturer of the device usually performs biocompatibility testing to evaluate the interaction between the device and tissues. Biocompatibility studies utilize analytical chemistry, in vitro tests, and animal models [30, 31]. Specific types of tests that might be performed on the device, by itself, include cytotoxicity in tissue culture, sensitization assays, irritation tests, acute and systemic toxicity tests, intracutaneous tests, implantation tests, and hemocompatibility tests.

For DNA-based vaccines, electroporation has been used to deliver the DNA into cells. Toxicity studies examining the specific method of electroporation are needed. Electropermeabilization may leave the target tissue damaged depending upon the electrical parameters associated with the electroporation [32]. For the technique to be clinically acceptable for use in gene/DNA delivery, there should be no permanent damage to the skin [33]. Electroporation devices that are intended for administration might need to be adjusted for use in animals; for example, the needle length of the injection array could be different in animal and human studies.

16.2.5 Considerations for Safety Assessment of Excipients, Residuals, and Contaminants

Excipients, such as buffer components and preservatives, are added to vaccine formulations to improve the stability of vaccine components. When selecting excipients for inclusion in vaccine formulations, it is preferable to use excipients where toxicology data are available and that have been previously used in other marketed vaccines and products, for which clinical safety has been already demonstrated. Such excipients should not be regarded as being "novel," and a scientific review of the available toxicology data would provide sufficient toxicology evaluation for the excipient. For novel excipients, toxicology studies are required. A study of the excipient within the repeat-dose toxicity studies for the vaccine is more relevant with respect to the interaction of the excipient with other vaccine formulation components.

Residuals and contaminants are substances that are used in the manufacturing process and may be present in the final formulation in residual amounts (e.g., formaldehyde, toxins, viral growth media). No specific regulatory guidelines are available for the safety assessment of vaccine residuals or contaminants. A determination or estimation of the "worst case" mass of the residuals or contaminant per vaccine dose could be made or measured directly if assays exist. ICH guidelines on impurities (ICH Q3A/B) do not cover biological or biotechnological products; however, the general principles of the guidelines could be applied [34, 35]. For example, ICH Q3B states that for drugs that are administered <1 g per day, the maximum reporting threshold for impurities or degradates should be 0.1% [35]. ICH Q3C on residual solvents discusses an approach for establishing permitted daily exposure (PDE) limits [36]. Both these concepts might be considered applicable to residuals and contaminants in vaccines.

The threshold of toxicological concern (TTC) concept that was developed for risk assessment of human exposure to even the most harmful of chemicals could be applied for excipients and residuals in vaccines. The goal of the establishment and application of acceptable TTC values was to avoid unnecessary toxicity testing and safety evaluations when human intake was below a threshold amount that would be safe even for harmful chemicals. In developing the TTC concept, an Expert Group under the International Life Sciences Institute (ILSI) considered a wide range of toxicological concerns including metabolism and accumulation, structural alerts,

endocrine disrupting chemicals, genetic toxicity, carcinogenicity, neurotoxicity, teratogenicity, developmental toxicity, allergenicity, and immunotoxicity [37]. This published work has been adopted by the European Medicines Agency for establishing a guideline on the limits of genotoxic impurities in pharmaceutical development [38]. Although the impurity guideline addresses TTC levels for potent genotoxic carcinogens and was not intended to be applied for residuals and contaminants, it could be an approach that is taken to address the levels of residuals and contaminants in vaccines.

16.3 Adequate Design of Toxicity Studies with Next Generation Vaccines

16.3.1 Approaches to Measurement of Immune Response in Toxicology Species

Animal species that are used in nonclinical safety studies with vaccine candidates should be able to mount an immune response, for example, antibody levels to the vaccine antigens. At the same time, toxicology studies are expected to be conducted in a laboratory animal species, for which historical control data exist to help distinguish true toxicity caused by the tested vaccine from potential background (not test article-treatment related) lesions occasionally found during a thorough histopathological examination of most organs and tissues involved in such studies. Rats are most commonly used in toxicology studies with a broad range of chemical entities and they are typically the species of choice for toxicity studies with vaccine candidates. However, rats are rarely used in nonclinical pharmacology studies that are focused on protective or therapeutic immune responses to a vaccine candidate. Instead, mice, rabbits and/or nonhuman primates (NHPs) are typical species in vaccine pharmacology studies based on available models of diseases and attempts to predict immune responses from animals to humans. In order to "bridge" the toxicology and pharmacology animal species, a measurement of immune responses in species selected for nonclinical safety evaluation (e.g., rats) to a vaccine candidate is included in a separate study or within a repeat-dose toxicity study [39]. This approach provides indirect evidence of the exposure and activity of the vaccine and is aligned with the general principle of all toxicology studies, in which the demonstration of the animal exposure to a test article following the administration of this test article in the course of a study is required. However, based on recent discussions and some regulatory guidelines [6], there are additional expectations (if not requirements) for toxicology studies of vaccines, that is, the animal species should be sensitive to the pathogenic organism or toxin targeted by the vaccine-induced immune response. Addressing this expectation in toxicology studies may be problematic when the "disease-sensitive species" are different from the "routine species" because the former are not well characterized to provide reliable data to

distinguish between "background" lesions relative to what may be considered a vaccine-related effect. Typically, a solution to this problem would involve the use of more than one species to evaluate safety of such vaccine candidate; for example, using a routine species in a well-controlled toxicity study (i.e., compliant with Good Laboratory Practice regulation) and a nonroutine species in an exploratory safety study, which is likely less comprehensive but with endpoints focused on pathogen-specific concerns [23]. In both types of studies, the immune response to the vaccine should be demonstrated.

The evaluation of the immune response to the vaccine relies on immunoassays that are developed in order to measure the most relevant endpoint, i.e. antibody response or cellular immune response. For the measure of specific antibodies, standard ELISA formats or multiplex assays for multiple antigens vaccine candidates are often applied [40]. When the candidate vaccine targets the cellular arm of the immune response, assays measuring cytokine-secreting antigen-specific T lymphocytes (such as γ-interferon ELISpot) can be utilized [41]. These assays are typically developed and performed to support nonclinical pharmacology studies (e.g., using mice, rabbits, or NHPs), and then are adopted for the use in toxicology selected species (e.g., rats).

16.3.2 Incorporation of Additional (Nonroutine) Endpoints in Toxicology Studies

Immune stimulation is an intended pharmacological effect of vaccines, and thus effects on various immune system parameters are expected and desirable. Such effects may include changes in hematology (various white blood cell types) and serum biochemistry (e.g., protein and globulin) parameters, local irritation and inflammation at the injection site, lymphoid enlargement and hyperplasia, and spleen weight increases [42]. These effects are generally modest and reversible, and, as consequences of the intended pharmacological activity of the vaccine, are usually not considered adverse.

Traditional vaccines containing aluminum salts as adjuvants have predominantly functioned via local rather than systemic mechanisms, and a systemic inflammatory response to these vaccines has generally not been a concern. However, as indicated above, the theoretical concern of synergistic immune stimulation seems to be heightened for novel, particularly "molecular" adjuvants such as Toll-like receptor (TLR) agonists [43] and cytokines [44, 45]. The safety concerns for the use of immunostimulatory adjuvants include potential excessive pro-inflammatory and pyrogenic responses (IL-6, TNFα, IL-8, IL-1β, PGE2); stronger or unexpected organ specific toxicity (local inflammation, cell death, immuno-dysregulation); severe local reactogenicity (increased vascular permeability, cellular infiltration, fluid accumulation); and break-down of self tolerance (dysregulation of T cells and other host cells). Therefore, the potential for a systemic inflammatory response for vaccines, particularly those containing

immuno-active adjuvants, is expected to be assessed within the nonclinical toxicology studies. While there are several examples of animal safety studies conducted with a novel adjuvant alone or included as a control group for the toxicology study with a vaccine containing that adjuvant, including oligonucleotides (e.g., CpG DNA sequences [46]), oil emulsions (e.g., MF59 [47]), and saponin-based (e.g., QS-21 [48]) adjuvants, they generally confirmed the expected dose-dependent effects based on mechanisms of action, but did not reveal any findings of toxicological concerns. In contrast, there are examples of unwanted immune responses in patients treated with experimental therapeutic vaccines in clinical trials. One example of adverse T cell-mediated toxicity induced by a therapeutic vaccine involved an amyloid-β vaccine AN1792, consisting of Aβ1-42 amyloid antigen and the QS-21 adjuvant, used in clinical studies for treatment of Alzheimer's disease [49]. Symptoms of meningoencephalitis that were observed in some trial patients were not predicted by nonclinical safety studies and the cause of the unwanted immune responses was difficult to establish. Based on some retrospective nonclinical studies using transgenic mouse models of the disease, it was suggested the inclusion of the QS-21 adjuvant in the AN1792 vaccine might have contributed to the adverse Th1 response, involving significant IFN-γ, IL-4, and TNF-α expression [50].

This example illustrates a great challenge we currently face in the development and safety evaluation of vaccine candidates, regarding the ability to prospectively identify potential overt immune stimulation in the presence of the desired immune responses to vaccines. A great effort is put in place by vaccine developers into the identification of biomarkers of adverse immune stimulation in nonclinical studies. A recent review of extensive studies on saponin-based adjuvant ISCOMATRIX™ [51] reflects this line of work and progress made in the characterization of both physicochemical properties and biological activity as well as markers of immune responses induced by this novel adjuvant. The described work on exploratory assessment of serum markers of auto-immunity, inflammation, and allergy is based on clinical studies with HPV16E6E7 and HCV Core vaccines containing ISCOMATRIX™. In these studies, measurements of anti-cardiolipin antibodies, anti-B2 glycoprotein 1 and IgE levels were evaluated. The results are not necessarily conclusive at this stage but help to direct future work on biomarkers in both animal and clinical studies.

Another line of current research on potential biomarkers of immune stimulation and inflammation includes genetic profiling in response to adjuvanted vaccines [52]. Metagene- and pathway-based analytical approaches were adopted to provide quantitative readouts with biological relevance which can be used to study mode of action and rank vaccine and adjuvant candidates under development [52]. Gene profiling was performed on peripheral blood from monkeys treated with several vaccines with known clinical adverse effects. A gene module data analysis approach was used to demonstrate that one of the gene modules could be used as a classifier to predict vaccine/adjuvant reactogenicity. The classifier gene set was then applied in subsequent monkey studies to predict reactogenicity associated with experimental vaccines.

While work focused on establishing reliable biomarkers to apply them to nonclinical and clinical safety evaluation is ongoing, some steps to address concerns about undesired immune stimulation in toxicology studies can be put in place by including additional parameters that are not routinely measured in these studies [53]. For example, potential prolonged systemic inflammatory responses can be assessed by measuring acute phase proteins, e.g. C-reactive protein (CRP), IL-6 levels, complement components and/or coagulation factors as part of enhanced clinical chemistry analysis of serum and/or plasma samples collected from vaccinated animals at appropriate time after the administration of the vaccine. When using an immune potentiating adjuvant, the development of anti-DNA or anti-RNA antibodies could be monitored. Potential pathogenic autoimmune responses against a particular tissue could be evaluated by targeted immunohistochemistry evaluation of this tissue.

16.4 Adequate Design of Developmental and Reproductive Toxicity Studies with Next Generation Vaccines

To date there is no documented evidence of reproductive toxic effects in humans caused by any approved vaccine. However, the regulatory authorities do not presume a product is safe until it has been directly tested using appropriate preclinical test methods and well-designed, adequately powered clinical trials [1]. Therefore, to address potential developmental hazards of vaccine candidates, developmental toxicity studies in animal models are currently required for vaccines indicated for maternal immunization and/or immunization of women of childbearing age, according to the FDA's guideline titled "Considerations for Reproductive Toxicity Studies for Preventive Vaccines for Infectious Disease Indications" [18].

16.4.1 Design According to Guideline Recommendations

Developmental and reproductive toxicity (DART) studies provide information on potential effects of the vaccine on fertility, fetal development, and postnatal development of the offspring [18]. Since the primary concern for preventive and therapeutic vaccines is safety during development and growth of the embryo and fetus, the evaluation is focused on effects on the pregnant/lactating female and embryo-fetal development following exposure of the female to the vaccine from implantation through the end of pregnancy, with follow-up of the offspring through weaning. A postnatal follow-up of the pups from birth to weaning is also included to assess normal growth, nursing activity, body weights, and viability which are established as reliable indicators of normal development. Design of vaccine DART studies has been reviewed by Wolf et al. [39] In brief, female animals are immu-

nized a few weeks before mating in order to ensure peak immune responses during the critical phases of pregnancy (e.g., organogenesis). Vaccine booster doses are then administered during gestation (embryo-fetal period) and lactation (postnatal period) to evaluate potential direct embryotoxic effects of the components of the vaccine formulation and to maintain an immune response throughout the remainder of gestation. If an adjuvant is included in the vaccine, an adjuvant-alone control group could also be included, similar to the approaches to general repeat-dose toxicity studies discussed above.

16.4.2 Considerations for Vaccines Containing Immune Potentiators

As for general toxicology studies, for next generation vaccines, and particularly vaccine containing immunopotentiating adjuvants, questions and concerns have been voiced regarding the design of DART studies as delineated in the FDA guidance. These questions were discussed at a workshop on nonclinical evaluation of vaccines [54]. It was reported that participants generally agreed that the primary objectives and design of current DART studies performed according to the existing guidelines are appropriate and no specific changes were recommended. Furthermore, it was confirmed that no specific immunotoxicological endpoints are necessary since the evaluation of antibody response to the vaccine antigen(s) in DART studies is adequate to assess an effect of the vaccine on the immune system in the treated mother and indirectly on the developing immune system of the offspring. Additional immune parameters should only be evaluated on a case-by-case basis where there is an increased concern for potential immunotoxicity. Also, if DART studies would reveal vaccine-induced adverse effects on either the pregnant/lactating animal, the embryo/fetal development or development of the offspring, further nonclinical studies to evaluate the cause of the effect should be conducted. Follow-up studies would include broader immunological evaluations, e.g. histochemical analysis for antibody depositions, evaluation of lymphoid organ weights, histology and hematology of the F1 generation.

16.5 Quality Tests for Biological Products and Cell Substrates

Quality control of biological materials involves analytical and biological testing to identify quality attributes such as identity, purity, potency, and mass, and assess safety including sterility, pyrogenicity, and adventitious agents. In vivo quality testing is conduced not only in support of marketed products but clinical materials as well as preclinical materials. The main goal of this type of testing is to identify issues that may have arisen during manufacturing (i.e., introduction of adventitious agents/contaminants, changes in potency and/or properties of cell substrates or other

biological starting materials over time). The testing may also be used as a screening tool for biological materials that may have inherent characteristics that may affect their safety and/or tolerability (e.g., pyrogenicity testing on a vaccine candidate with bacterial components).

Potential sources of contamination of biotechnology products include the original source of the cell lines or from adventitious introduction during the manufacturing process. Some examples of these sources of contamination (as listed in ICH Q5A [22] and USP <1050> [55]) include viruses introduced into the Master Cell Banks (MCB) via: (1) derivation of cell lines from infected animals; (2) use of virus to establish the cell line; (3) use of contaminated biological reagents such as animal serum components; and (4) contamination during cell handling. In the case of the introduction of adventitious viruses, sources include: (1) the use of contaminated biological reagents such as animal serum components; (2) the use of a virus for the induction of expression of specific genes encoding a desired protein; (3) the use of a contaminated reagent, such as a monoclonal antibody affinity column; (4) the use of a contaminated excipient during formulation; and (5) contamination during cell and medium handling. Monitoring of cell culture parameters can be helpful in the early detection of potential adventitious viral contamination.

16.5.1 Regulatory Guidelines for Quality Testing

General guidelines available for this type of testing include the European Pharmacopoeia (EP [56]), the United States Pharmacopeia (USP [57]), the United States Code of Federal Regulations (US CFR [58]) and the Product License. Other countries may also have specific Pharmaopoeia (e.g., China, Japan, Brittan) so manufacturers should also consult these guidelines prior to marketing vaccines in these regions. Typically, the details of the testing contained in the Product License supersede the most current guidance documents unless specific regulatory approvals are sought to update the license in question. For more specific guidance for particular types of vaccines, cell substrates, or stages of production, additional guidelines are available (Table 16.1). Guidance documents are also available for Regulatory Submissions [59]. Testing supporting release of product generally follows current Good Manufacturing Processes (cGMP) guidelines [60, 61] though some tests are conducted per GLPs (e.g., tumorigenicity testing).

Testing requirements vary by region so manufacturers who market biologics worldwide typically design the assays such that the criteria for all markets may be satisfied in a single assay. For example, the specifications for the General Safety test listed in the CFR [62] differ from those in the EP [63]. Therefore, one could design the general safety test to satisfy all markets by using the greatest number of animals specified (5 mice and 2 guinea pigs) and a weight range inclusive of the ranges in all regions (17.0–21.9 g for mice and 250–350 g for guinea pigs). The duration is the same for both regions but if one were longer, presumably the longer duration would be selected. If the specifications selected are outside the range for one of the markets, it may be necessary to gain regulatory approval based on the rationale of reduction in animal use.

16.5.2 In Vivo Quality Control Tests

The specific in vivo tests include various species, as each species is more or less sensitive to particular adventitious agents and the most sensitive species should always be used. Adult and suckling mice are utilized to detect adventitious viruses. Adult mice detect lymphocytic choriomeningitis virus (LCMV), coxsackieviruses, flaviviruses, and rabies virus. Suckling mice detect coxsackievirus types A and B and other picornaviruses such as polioviruses and echoviruses, alphaviruses, bunyaviruses (including phleboviruses and nairoviruses), arenaviruses, flaviviruses, rabies, and herpesviruses (such as herpes simplex virus). Guinea pigs are sensitive to *Mycobacterium tuberculosis* and adventitious viruses including paramyxoviruses (including Sendai virus), reoviruses, and filoviruses, and rabbits are used to screen for simian Herpes B virus. Eggs are also utilized via various injection routes for the detection of herpesviruses, poxviruses, rhabdoviruses, rickettsiae, mycoplasmas, bacteria, orthomyxoviruses (influenza virus), and paramyxoviruses (mumps, measles, parainfluenza viruses), alphaviruses, and vesiculoviruses. In the antibody production test, hamsters, rats and mice are utilized to detect specific viruses. The hamster antibody production (HAP) test is utilized to detect lymphocytic choriomeningitis virus (LCMV), pneumonia virus of mice (PVM), reovirus type 3 (Reo3), Sendai virus, and simian virus 5 (SV5). The rat antibody production (RAP) test is specific for Hantaan virus, Kilham rat virus (KRV), LCMV, mouse adenovirus, mouse encephalomyelitis virus (Theilers, GDVII), PVM, rat coronavirus (RCV), Reo3, sialodacryoadenitis virus (SDAV), Sendai virus, and Toolan virus (HI). The mouse antibody production (MAP) test detects Ectromelia virus, mouse rotavirus (EDIM), Hantaan virus, LCMV, lactic dehydrogenase virus (LDM), minute virus of mice (MVM), mouse adenovirus (MAV), murine cytomegalovirus (MCMV), mouse encephalomyelitis virus (Theilers, GDVII), mouse hepatitis virus (MHV), PVM, polyoma virus, Reo3, Sendai virus, thymic virus, and K virus [20].

The endpoints vary by test and include physical signs, survival, body weight, body temperature, antibody levels, and/or gross necropsy and/or histopathological evaluation (Table 16.3).

16.5.3 Alternatives for In Vivo Release Tests

16.5.3.1 In Vitro Alternatives

Alternatives for some of the in vivo tests have been developed. For example, in vitro alternatives to the Rabbit Pyrogen Test include the Limulus amebocyte lysate (LAL) and the Monocyte Activation Test (MAT). The LAL assay is a well-established in vitro test widely used for the detection of pyrogenic endotoxins in biologic products. While this assay has utility for its intended purpose, it is unable to detect non-endotoxin pyrogens and false positive results may be obtained for vaccines that contain bacterial components. Therefore, the rabbit pyrogen test is still used for all products for

Table 16.3 In vivo quality control tests

Purpose	Test	Products tested	Endpoint	Method	Acceptance criteria
Test for extraneous contaminants	General safety/ abnormal toxicity[a]	Final product	Physical signs/ survival/body weight	At least 2–5 mice and 2 guinea pigs are injected IP in a volume of 0.5–1 or 5 mL for mice and guinea pigs, respectively. Animals are observed daily for 7 days [62, 63]	EP: none of the animals die or show signs of ill health. If >1 animal dies, the preparation fails. If no more than one animal fails to meet these criteria, the test may be repeated once US CFR: (1) they must survive the test period, (2) they do not exhibit any response which is not specific for or expected from the product and which may indicate a difference in its quality and (3) they weigh no less at the end of the test period than at the time of injection. The test may be repeated twice, in the species that failed only, if any of the acceptance criteria are not met with the caveat that the second repeat test must include twice the number of animals in the first repeat test
Purity	Pyrogen test	Final product	Body temperature	Rabbit body temperatures are monitored for 3 h at intervals not more than 30 min following IV injection of the product into three rabbits. Temperature rise is determined by the difference between the maximum temperature rise versus the initial/control temperature [64–66]	USP: if any of the three initial rabbits have a body temperature equal to or exceeding 0.5°C, the test is repeated in five additional rabbits. The test passes provided the sum body temperature increase of all eight rabbits does not exceed 3.3°C US CFR: per the USP with the exception that if the material requires testing in five additional rabbits, the test passes if not more than three of the eight rabbits show individual rises in temperature of 0.6°C or more, and if the sum of the eight individual maximum temperature rises does not exceed 3.7°C EP: the test passes if the cumulative temperature in the three rabbits does not exceed 2.65°C. If the initial test does not pass, further groups of 3 rabbits are tested up to a total of 12 rabbits. The test fails if the cumulative temperature rise in all 12 rabbits is greater than 6.60°C

Adventitious agents	Adult and suckling mice	Cell banks and vaccine seeds/ bulks	Survival, evidence of transmissible agent or other viral infection	No less than 10–20 adult mice are injected IP and also IC in some cases or IM only and animals are observed for 21–28 days. No less than 10–20 suckling mice are injected IP and also IC in some cases or SC only and animals are observed for 14–28 days. If any animals die or show signs of disease after the first 24 h, autopsy and in some cases prepare and inject a suspension of appropriate tissues from the first group of animals into a second group of animals. In some countries a suspension of appropriate tissues from all survivors of the suckling test is injected into a second group of animals [20, 21, 55, 67]	At least 80% of inoculated mice must survive and show no evidence of infection attributable to the test material
	Guinea pigs			Five guinea pigs are injected IP and in some cases also IC and observed for 42 days to 6 weeks. Any animals that die or show signs of disease after the first 24 h are autopsied and all surviving animals are autopsied at the end of the test. Tissues are evaluated for signs of infection attributable to the test article [20, 21, 67]	
	Rabbits			Five rabbits are inoculated with product ID and also SC in some cases and observed for 4 weeks. Animals that die or show signs of sickness are investigated for cause of death via necropsy [20, 21]	
	Embryonated chicken eggs		Survival	Two routes of administration are utilized for the egg test. 10 eggs each are inoculated via yolk sac and the allantoic route. Eggs are incubated for not less than 5 or 9 days and evaluated for hemagglutinins. In some countries it is also required that passage of allantoic fluids and yolk sacs into a second group of eggs be conducted [20, 21, 55, 67, 68]	The test is valid if greater than 80% of test and control eggs are free of hemagglutinins and do not show gross evidence of viral infection.
	Antibody production tests: performed when potential exists for exposure to rodent viruses.	Cell banks	Antibodies to species-specific agents	The test article is inoculated into specific-pathogen-free (SPF) animals that are subsequently tested for antibodies to specific agents. Hamsters, rats, and mice are utilized as each detects multiple, specific viruses. [20, 21, 55, 68]	Absence of detectable antibodies to specific viruses

(continued)

Table 16.3 (continued)

Purpose	Test	Products tested	Endpoint	Method	Acceptance criteria
Cell properties	Tumorigenicity[b]	Cell banks	Tumor formation	Ten nude (nu/nu) mice or other suitable animals are inoculated SC or IM with cells at or beyond the end-of-production passage level. A group of animals inoculated with positive (e.g., HeLa) control cells is included. Animals are observed for a minimum of 16 weeks, up 7 months in some cases. An interim necropsy is included in some cases. Endpoints include tumor formation and gross and histological evaluations [20, 21, 68]	A test fails if progressive tumor formation with histopathic or genotypic confirmation of inoculated cells is observed at the site of injection in at least 2 out of 10 animals. At least 9 out of 10 animals injected with positive control cells should show progressively growing tumors in order for test to be valid
	Oncogenicity. To assure that agents that could immortalize cells and endow them with the capacity to form tumors are not present in cell substrate evaluated	Lysates of cell banks	Tumor formation	Typically test cellular DNA and cell lysates in newborn nude mice, newborn hamsters, and newborn rats. FDA/CBER and other regulatory agencies can be consulted for methods [20]	

Neurotropisms	Neurovirulence[c]	Viral vaccine seeds/ bulks	CNS effects	Not fewer than ten suitable *Macaca* or *Cercopithecus* monkeys are inoculated with 0.5 mL of material to be tested in thalamic region of each hemisphere. Control monkeys included as cage-mates or in immediate vicinity. Inoculated monkeys are observed for 17–21 days. At the end of observation period, autopsy and histopathological examinations of appropriate areas of brain are evaluated for evidence of CNS involvement [69]	Material complies with test if no unexpected clinical or histopathological evidence of involvement of CNS attributable to inoculated virus
Potency[d]	Immunogenicity	Final product	Vaccine titer	Product-specific [70]	Product-specific

IP intraperitoneally, *IC* intracerebrally, *SC* subcutaneously, *IV* intravenously, *IM* intramuscularly, *ID* intradermally, *US CFR* United States Code of Federal Regulations, *EP* European Pharmacopoeia, *USP* United States Pharmacopeia

[a]It should be noted that some products elicit expected physical signs based on components in the test sample itself so the dose and/or duration may be altered to allow for reversal of the expected signs before assessing for extraneous contaminants. In addition, some products may be exempt from the test based on the mode of administration or nature of the product

[b]In general diploid cell lines are considered to have low potential for tumorigenicity and well-established cell lines may not require repeated testing

[c]Neurovirulence testing is required, for example, if inadequate data on neurovirulence of a virus for which a novel vaccine is being developed, if neurotropism or neurovirulence is apparent, or if a novel vaccine has been attenuated by passage in neuronal tissue

[d]Tests may be conducted in vivo and/or in vitro

which the LAL is inappropriate. In an effort to reduce animal use, the MAT, a novel in vitro assay has been developed and validated for the detection of both endotoxin and non-endotoxin pyrogens [71]. The assay involves the stimulation of a lymphocyte population in whole blood, peripheral blood mononuclear cells (PBMCs), or a reproducible cell line (MonoMac 6) by the analyte of interest and the measurement of cytokine release (IL-6, IL-1β, TNF-α). This assay is also listed as an alternative to the rabbit pyrogen test in the EP [72].

Some of the tests for adventitious agents also have in vitro alternatives. In vitro tests such as culture and PCR for the identification of *Mycobacterium* are, in some cases, acceptable as an alternative to the test in guinea pigs and the test in rabbits for the presence of herpes B virus in primary simian cultures may be replaced by a test in rabbit kidney cell cultures [20, 21].

It is also possible to replace in vivo potency tests with in vitro methods. The design of potency studies is flexible and generally product-specific and if an in vivo model is available, one may start with an animal (typically mouse) test with plans to move to an in vitro model proven to correlate with in vivo data in an effort to reduce animal use. It should be noted, however, that in vitro tests may not always correlate with clinical experience due to their ability to detect chemical changes that may not lead to functional effects on potency.

16.5.3.2 In Vivo Alternatives

In vivo assays designed to detect neurovirulent potential in live virus vaccines have traditionally required the use of nonhuman primates (NHPs) in the Monkey Neurovirulence Test (MNVT). While this type of test remains appropriate for some neurotropic virus strains (e.g., polio and yellow fever), at a workshop in 2005 jointly organized by the International Association for Biologicals (IABs), the EP and the WHO, it was recommended that MNV testing no longer be required for established strains with proven safety records such as measles, mumps, rubella, and varicella [73]. In fact, the EP monographs for measles [74], mumps [75], rubella [76], and varicella [77] now require that only new strains of these viruses be evaluated for neurovirulence and that the test, in an appropriate animal model, be conducted during preclinical development only. In addition, FDA has removed the requirement for neurovirulence testing from the CFR [73]. In some cases, neurovirulence testing is required, for example, if inadequate data on neurovirulence of a virus for which a novel vaccine is being developed, if neurotropism or neurovirulence is apparent, or if a novel vaccine has been attenuated by passage in neuronal tissue. However, novel models for neurovirulence testing that do not involve the use of NHPs are being pursued. For example, Rubin et al. have developed a neonatal rat model which has shown better predictive value than the MNVT in distinguishing between neurovirulent and attenuated strains of mumps virus [78]. Other test systems such as marmosets have also been evaluated and show some promise. Nonanimal-based testing methods have also been considered but full replacement of animal testing may not be feasible as the complexity of neurovirulent viruses may not be adequately reflected [79].

16.5.4 In Vitro Quality Tests

In addition to the in vivo tests described in Table 16.3, there are several in vitro tests that also detect adventitious viruses. The Cell Culture Safety Test in human diploid or monkey kidney cells detects a variety of adventitious viruses that include cytopathic viruses, hemadsorption viruses, and hemagglutinating viruses. The use of Human Diploid Cells identifies a variety of human viruses (such as herpesviruses, adenoviruses, coronaviruses, reoviruses, alphaviruses, rubella, flaviviruses, rabies, enteroviruses, certain strains of hepatitis A virus, poliovirus, coxsackie B virus, echovirus, rhinoviruses, orthomyxoviruses, paramyxoviruses) and simian viruses (such as simian cytomegalovirus). The use of Monkey Kidney Cells could identify human viruses [such as enteroviruses, coxsackie B viruses, echoviruses, orthomyxoviruses, paramyxoviruses, HSV, poxviruses, polyomaviruses, rotavirus, alphaviruses, rubella, flaviviruses, rabies viruses, vesiculoviruses, filoviruses, influenza viruses, bunyaviruses (including phleboviruses and nairoviruses), arenaviruses, and reoviruses, polioviruses, rhinoviruses, adenoviruses (some strains)] and simian viruses (such as herpes B virus) [20].

Other tests for detection of adventitious viruses include: (1) Transmission Electron Microscopy (TEM) which can detect viral particles in a cell substrate, including those from endogenous retroviruses; (2) Reverse transcriptase (RT) assays which can detect any retrovirus, as all retroviruses encode and contain RT; (3) Infectivity Tests for retroviruses which can be performed on a case-by-case basis; and (4) PCR or Southern Blot which can be performed to detect specific viruses. In vitro tests for nonviral adventitious agents include tests for mycoplasma, mycobacteria, and bacterial and fungal sterility.

Other safety tests for product release include tests for the presence of residual cells and DNA. Residual cellular or nuclear material in the final product poses a potential risk because of oncogenic and/or infectivity potential. DNA can be removed, digested, or inactivated to lessen these risks.

16.6 Conclusion

The development of a broad range of novel or next generation vaccines containing more synthetic and/or recombinant components rather than microorganism-derived components has a clear advantage from the manufacturing and process control perspective. With the continuous progress in technology, especially molecular and genetic methodologies applied both in discovery of vaccines and formulation sciences, the development of well-defined specific antigens for use in vaccines enables production of next generation novel vaccines to prevent and/or treat diseases which have been refractory to vaccination in the past. Scientific and technological advances have led to improved vaccine products aiming at elimination of potentially virulent or carcinogenic components and reduction of impurities.

However, vaccines containing "purer" antigens are often not very immunogenic and therefore require addition of more effective adjuvants and other excipients. Paradoxically, while the next generation vaccines are more defined and better characterized than the traditional vaccines, they are viewed as "less natural." Also, advances in molecular engineering leading to the use of novel adjuvants and other components as well as novel delivery systems are not always paralleled by a full understanding of biological mechanisms of action of these components. A limited understanding of mechanism of action of newly available vaccine adjuvants leads to increased concerns about their safety [3]. Nevertheless, many recent scientific and regulatory discussions dedicated to this topic seem to indicate that currently recommended and applied approaches to nonclinical development, including toxicology studies, of vaccine candidates are appropriate and adequate [54]. The principles for the nonclinical safety assessment reviewed in this chapter should provide a foundation for the evaluation of next generation vaccines.

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