REVIEW

Overview of Vaccines

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Abstract

This article lists the vaccines current available for the control of both viral and bacterial infections. They may be attenuated live or inactivated whole microorganisms, or subunit preparations. Many more are in the pipeline and increasing attention is being given to establishing their safety before registration. Following the earlier eradication of smallpox, good progress is now being made toward the global eradication of poliomyelitis and a new program to eliminate measles from the Americas has begun. A variety of new approaches to vaccine development is now available. The hepatitis B virus surface antigen, made by DNA-transfected yeast or mammalian cells, is the basis of the first genetically engineered vaccine. Early in the 21st century, new vaccines based on oligopeptides, recombinant live viral or bacterial vectors (often existing live vaccines), or recombinant DNA plasmids are likely to be registered for human use. The efficacy of vaccines depends on the immune responses generated, and the recent substantial increase in our understanding of the mammalian immune system now offers great opportunities for manipulation to best obtain desired responses. These include mixing vaccine formulations to maximize immune responses, and combining vaccines to simplify their administration. Despite these advances, some persisting infections, such as those caused by HIV, plasmodia, and mycobacteria, still pose a great challenge to vaccine developers.

Index Entries: Vaccines; vaccination; safety; efficacy; disease eradication; immune response manipulation; oligopeptides; chimeric live vectors; DNA plasmids; vaccine administration.

1. Patterns of Infectious Processes

Most vaccines are designed as a prophylactic measure, that is, to stimulate the immune response so that on subsequent exposure to the particular infectious agent, infection is either prevented or the extent of infection is so low that clinical disease does not occur. There is also increasing interest in designing vaccines for use as a form of immunotherapy, for infections such as HIV and especially for noncommunicable diseases such as cancer and autoimmunity. This article discusses only prophylactic vaccines.

1.1. Intracellular vs Extracellular Patterns of Infection

There are two contrasting types of infectious processes. Some organisms (including all viruses), bacteria, and parasites replicate inside susceptible cells. Other bacteria and parasites only replicate extracellularly. The malaria parasite has an intracellular phase as one part of its life cycle because of this difference, the relative contributions of the different immune responses required to control the infection will differ.

1.2. Acute vs Persisting Infections

In the case of acute infections, exposure of a naive individual to a sublethal dose of the infectious agent may cause disease, but the immune response so generated will clear the infection within days or a few weeks. Death may occur if the infecting dose is so high that the induced immune response is qualitatively or quantitatively insufficient to prevent continuing replication so that the host is overwhelmed.

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Table 1
Currently Registered Viral and Bacterial Vaccines for Human Use

Viral	Bacterial	Other
Live attenuated		
Vaccinia (smallpox)	BCG	
Polio (OPV)	Salmonella (Ty21a)	
Measles		
Adeno[a]		
Mumps		
Rubella		
Yellow fever		
Herpes zoster		
Inactivated whole organism		
Influenza	*Vibrio cholerae*	*C. burnetii*
Rabies	*Bordetella pertussis*	
Japanese encephalitis	*Yersinia pestis*	
Polio (IPV)		
Hepatitis A		
Subunit		
Hepatitis B	*Streptococcus pneumoniae*	
Influenza	*Salmonella typhi*	
	Haemophilus influenzae, type b	
	Acellular *B. pertussis* (aP)	
Conjugates (Polysaccharide protein carrier)	*H. influenzae*, type b	
Toxoids	*Clostridium tetani*	
	Corynebacterium diphtheriae	
Combinations		
Measles, Mumps, Rubella	Diphtheria, pertussis, tetanus (DPT) DaPT	
	DPT, *H. influenzae* type b (Hib)	

[a]For US defence force personnel only.
[b]General information, *see* **ref. *43***.

In contrast, many infections—viral, bacterial, and parasitic—persist for months or years particularly if the process of infection by the agent results in the evasion or subversion of what would otherwise be an effective immune-control response. Some of the evasive mechanisms employed (described in **ref. *1***) are remarkably ingenious.

Most of the vaccines registered for medical use in developed countries and discussed briefly in the next selection, are designed to prevent/control acute infections in humans.

2. Types of Vaccines

Almost all of the vaccines in use today are against viral or bacterial infections (**Table 1**). They are of three types—live attenuated microorganisms, whole inactivated microorganisms, and subunit preparations.

2.1. Live, Attenuated Microorganisms

Many regard some of the live attenuated viral vaccines as the most successful of all vaccines, with one or two administrations conferring long-lasting immunity. Four general approaches to develop such vaccines have been used:

1. One approach, pioneered by Edward Jenner, is to use a virus that is a natural pathogen for another host as a vaccine in humans. Examples are the use of cowpox and parainfluenza viruses in humans and the turkey herpes virus in chickens. More recently, the use of avipox viruses, such as fowlpox and canarypox, which undergo

an abortive infection in humans, has given encouraging results in human trials *(2)*.

2. The polio, measles, and yellow fever vaccines typify the second approach. The wild-type viruses are extensively passaged in tissue culture/animal hosts until a balance is reached between loss of virulence and retention of immunogenicity in humans.
3. Type 2 polio virus is a naturally occurring attenuated strain that has been highly successful as a vaccine. More recently, rotavirus strains of low virulence have been recovered from children's nurseries during epidemics *(3)*.
4. A fourth approach has been to select mutants that will grow well at lower than body temperatures but very poorly above 37°C. The cold-adapted strains of influenza virus grow at 25°C and have mutations in four of the internal viral genes *(4)*. Although such strains were first described in the late 1960s, and have since undergone extensive and successful clinical trials in adults and children in the United States and Russia, they are not yet registered for human use in the United States, though they have been used in Russia for many years.

In contrast to the many successful attenuated viral vaccines, BCG (Bacille Calmette-Guérin) for the control of tuberculosis remained, until comparatively recently, the only registered live attenuated bacterial vaccine. Though still widely used in the World Health Organization Expanded Program for Immunization (WHO/EPI) for children; especially in developing countries, it has given very variable results in adult human trials. However, prolonged studies to make other attenuated bacterial vaccines, especially against *Salmonella* infections, have led more recently to a general approach involving the selective deletion or inactivation of groups of genes *(5,6)*. The first success was with the strain Ty21a, which has a faulty galactose metabolism, the success of which led to the development of strains with other gene deletions *(6)*.

This approach also shows promise for complex viruses. Thus, 18 open reading frames have been selectively deleted from the Copenhagen strain of vaccinia virus, including six genes involved in nucleotide metabolism, to form a preparation (NYVAC) that is of low virulence, but retains immunogenicity *(7)*. The selective deletion of specific nucleic-acid sequences is also being tried with simian immunodeficiency virus with some initial success *(8)*.

This approach offers the prospect of a selective and reproducible means of producing adequately attenuated viral and bacterial preparations. Live, attenuated viral and bacterial vaccines have the potential of stimulating the widest range of different immune responses, with the greatest chance of either preventing, or controlling and clearing a later infection by the wild-type agent.

2.2. Inactivated Whole Microorganisms

Inactivation of viruses such as polio, influenza, rabies, and Japanese encephalitis viruses, and some bacteria, including *Bordetella pertussis* and *Vibrio cholerae*, is the basis of vaccines that have varying efficacy. Compared to the attenuated preparations, these vaccines need to be administered in substantially larger doses and sometimes repeatedly. The viral vaccines are generally effective in preventing subsequent disease; the low efficacy (approx 70%) of the influenza viral vaccine is in part, owing to the continuing antigenic drift to which this virus is subject. In contrast, the *B. pertussis* vaccine, which is quite effective, is being replaced by a subunit vaccine in many developed countries because of adverse effects attributed to vaccination with the whole-cell vaccine *(9)*. The current *V. cholerae* vaccine may be replaced either by a later, rather similar preparation administered with a mucosal adjuvant *(10)*, or by a live, attenuated preparation *(5)*.

Inactivated whole-organism vaccines generally induce many of the desirable immune responses, particularly infectivity-neutralizing antibody, but often do not generate a class I major histocompatibility complex (MHC)-restricted cytotoxic T-lymphocyte (CTL) response, which has been shown to be the major response required to clear intracellular infections by viruses, some bacteria, and some parasites. However, γ-irradiated influenza virus *(11)* in contrast to ultraviolet (UV)-irradiated influenza virus *(12)*, and inactivated feline immunodeficiency virus administered with a certain adjuvant *(13)*, generate strong CTL responses.

2.3. Subunit Vaccines

The generation of antibody that prevents infection by both intra- and extracellular infectious agents has been regarded as the prime requirement of a vaccine. The epitopes recognized by such antibodies are most usually confined to one or a few proteins or carbohydrate moieties present at the external surface of the agent. Isolation (or synthesis/biosynthesis) of such components formed the basis of tine first viral and bacterial subunit vaccines. Viral vaccines were composed of the influenza viral surface antigens (the hemagglutinin and neuraminidase), and the hepatitis B surface antigen (HBsAg). Bacterial vaccines contained the different oligosaccharide-based preparations from encapsulated bacteria. In the latter case, immunogenicity was increased especially for infants by coupling the haptenic moiety (carbohydrate) to a protein carrier, thereby ensuring the involvement of helper T-cells (Th cells) in the production of different classes of immunoglobulin (Ig), particularly IgG. The two bacterial toxoids, tetanus and diphtheria, represent a special situation where the primary requirement was neutralization of the activity of the toxin secreted by the invading bacteria.

HBsAg is present as such in the blood of hepatitis B virus-infected people. This was the source of the antigen for the first vaccines and this remains the case for the vaccine used in third-world countries. A major advance occurred when the same product was made from yeast cells transfected with DNA coding for this antigen, initiating the era of genetically engineered vaccines *(14)*. Up to 17% of adults receiving this vaccine are either poor or nonresponders, partly because of the age of the recipients, but mainly because of their genetic makeup *(15)*.

3. Vaccine Safety

All available data concerning the efficacy and safety of a candidate vaccine are reviewed by regulatory authorities before registration. At that stage, potential safety hazards, which occur at a frequency of perhaps 1/10,000, are likely to have been detected. There are examples of undesirable side effects occurring at much lower frequencies, which are seen only during immunosurveillance following registration, but these may be so low and delayed that their occurrence as a consequence of vaccination is difficult to prove. For example, following the mass vaccination program of people in the United States with a swine influenza vaccine in 1976–1977, a small proportion developed Guillain-Barre syndrome *(16)*. This turned out to be an isolated event.

In the prolonged absence of frequent outbreaks of disease by vaccine-preventable infections because of successful vaccination campaigns, the occurrence of low levels of undesirable side effects following vaccination may gain notoriety. The evidence bearing on causality and specific adverse health outcomes following vaccination against a number of childhood viral and bacterial infections, mainly in the United States, has recently been evaluated by an expert committee for the Institute of Medicine in the United States *(17)*. The possibility of adverse neurological effects was of particular concern, and evidence for these as well as several immunological reactions, such as anaphylaxis and delayed-type hypersensitivity (DTH), was examined in detail. In the great majority of cases, there was insufficient evidence to support a causal relationship, and where the data were more persuasive, the risk was considered to be extraordinarily low.

Measles vaccination has provided an interesting example of vaccine safety. The WHO/EPI has provided data illustrating the remarkable safety of the standard vaccine *(18)*. Furthermore, although natural measles infections induces an immunosuppressive state from which most children recover, the aforementioned study *(17)* recorded only two cases of immunosuppression in immunocompromised children following vaccination. In many developing countries, measles vaccination is given at 9 mo of age. This delay is necessary to allow a sufficient decay of maternally acquired antibody. This decay to low levels occurs earlier in some infants, allowing the opportunity for infection by circulating wild-type virus before 9 mo of age. This factor contributes significantly to the 1–2 million deaths/yr from measles infections world-wide. To lessen this risk, "high-titer"

measles vaccines were developed that could be effective in 5–6 mo old children in endemic regions. Trials in several countries showed their apparent safety and ability to induce satisfactory immune responses in this age group, so their general use was authorized by the WHO in 1989. Unfortunately, reports shortly appeared recording unexpected cases of mortality following vaccination, especially in young girls in disadvantaged populations *(19)*, leading to the withdrawal of these vaccines from use. It seemed likely that the high-titer vaccine caused a degree of immunosuppression sufficient to allow serious infections by other agents to occur.

Inactivation of a whole microorganism, even a relatively simple virus, does not guarantee safety. Immunization of infants with inactivated measles or respiratory syncytial virus (RSV) preparations sensitized some recipients to severe reactions when they were later exposed to the wild-type virus *(20)*. Nevertheless, the great safety record of the subunit viral vaccines is one factor contributing to the attractiveness of the subunit approach to vaccine development.

4. Efficacy

There could be no more persuasive evidence of the worth of an immunization program as a very effective public health procedure than the eradication/elimination of an infectious agent. Global eradication of a disease agent was first achieved in 1977 when the last case of endemic smallpox was detected, slightly more than 10 yr after the intensified WHO campaign was initiated. Following intensive immunization campaigns, poliomyelitis was declared eliminated from the Americas in 1994, 3 yr after the last case of endemic polio in the Americas was detected *(21)*. Endemic poliomyelitis has now also been eliminated from the Western Pacific region and good progress is being made in Africa. Clearly, the smallpox and poliovirus vaccines were/are highly efficacious, although both elicited undesirable side effects *(22,23)*. The achievements toward polio elimination are remarkable in view of the difficulties involved compared to smallpox *(24)*. The success with polio in the Americas has led to the next challenge in that region—the elimination of measles, another viral infection specific for humans, from the Americas by the year 2000 *(25)*.

These achievements, together with the emergence of diseases such as AIDS, have greatly increased interests in all aspects of "Vaccinology." The following sections discuss the need for improved and new vaccines against a variety of infectious agents, some of the new approaches now available for vaccine development, the properties and functions of different immune responses, and some of the obstacles that still face the vaccine developer.

5. Opportunities for Improved and New Vaccines

There are two opportunities for further vaccine development—improved vaccines to replace some existing vaccines, and new vaccines against the many infectious agents that still cause considerable morbidity, and in some cases mortality, especially in developing countries. **Table 2** lists examples of diseases where improved vaccines are desirable, and some viral, bacterial, and other infections for which vaccines are not yet available.

The rationale of the need for improved compared to current vaccines varies. For example, despite the efficacy and safety of the standard measles vaccine, there is the need for a modified or new vaccine that would be effective in the presence of maternal antibody. A genetically more stable type 3 live polio virus and a means to make the oral polio vaccine and other live vaccines more heat-stable is highly desirable. The standard Japanese encephalitis viral vaccine produced from infected baby mouse brains needs to be replaced, and a live attenuated virus preparation used in China shows encouraging efficacy.

Above all, progress toward fulfillment of the aim of the Children's Vaccine Initiative, i.e., to produce a formulation of children's vaccines that can be administered at a single visit at or near birth and provide effective immunity against numerous diseases *(26)*, is likely in the long term to result in major changes.

Vaccines against many of the other agents in **Table 2** are unlikely to be made using traditional

Table 2
Opportunities for Improved and New Vaccines

Improved	New
Viral	
Influenza A and B	Corona
Japanese encephalitis	Cytomegalo
Polio	Dengue
Rabies	Hepatitis C
Measles	HIV 1 and 2
	Hantan
	Herpes
	Norwalk agent
	Papilloma
	Parainfluenza
	Respiratory syncytial
	Rota
	Varicella
Bacterial	
V. cholerae	Chlamydia
Meningococcus	*E. coli*
M. tuberculosis	Group A and B streptococcus
B. pertussis	*Haemophilus ducreyi*
	M. leprae
	Meningococcus B
	Neisseria gonorrhoeae
	Shigella
Others	Malaria
	Schistosomiasis
	Giardia
	Filariasis
	Treponema
	B. burgdofferi

techniques. For example, *M. leprae* cannot be produced in sufficient quantity to make a whole organism vaccine to administer to >100 million people. It may also be impracticable to produce large quantities of some viruses to form the basis of a vaccine. But above all, some of the new approaches to develop vaccines hold out so much promise that they are bound to influence future manufacturing practices greatly.

6. New Approaches to Vaccine Development

There are basically three new approaches.

1. The use of anti-idiotype antibody preparations to mimic epitopes that react with B-cell Ig receptors;
2. The synthesis of oligo/polypeptides, which reflect naturally-occurring amino-acid sequences in protective proteins of the infectious agent;
3. The use of recombinant DNA (rDNA) technology to obtain DNA/cDNA coding for antigen(s) of different infectious agents or other factors such as cytokines, and to use these mainly in three different ways:
   a. To transfect cells so that the inserted DNA/cDNA is translated and expressed;
   b. To insert the DNA/cDNA into the genome of other viruses or bacteria, which are usually chosen as vectors because of their record as effective and essentially safe vaccines; such chimeric constructs are potential new vaccines, and;
   c. A plasmid containing the DNA/cDNA can be directly injected into cells in vivo, where it is translated and expressed and immune responses initiated.

6.1. Anti-Idiotypes

The attractions of this approach include the facts that the preparation should mimic peptide, protein, or carbohydrate epitopes; and the conformation of the epitope in question. Despite such advantages, this approach has not prospered, and is, unlikely to have a significant impact in the foreseeable future.

6.2. Oligo/Polypeptides

The sequences chosen may contain either B-, T-, or both B- and T-cell epitopes and determinants. Sequences containing B-cell epitopes may be conjugated to carrier proteins that frequently act as a source of T-cell determinants or are assembled in different configurations to achieve particular conformations or produce multiple determinants. An obvious advantage of this approach is that the final product contains the protective sequences of the antigen and eliminate sequences that might mimic those of any host antigen. Multimeric constructs, such as Multiple Antigenic Peptide Systems (MAPS), can be highly immunogenic *(27)*. Further, the immunogenicity of important, but "cryptic" sequences may sometimes, be enhanced by deletion of other, more dominant sequences of a molecule *(28)*, and this may be use-

ful if the latter sequences are especially subject to mutation. New methods of oligopeptide synthesis offer the possibility of more closely mimicking the conformation in the original protein *(29)*.

This is now a very active field, especially in recent studies aimed at inducing CTL responses to tumors. Peptide-based vaccines seem assured of a share of the future vaccine market.

6.3. Transfection of Cells with DNA/cDNA

Three cell types have been used—prokaryotes; lower eukaryotes, mainly yeast; and mammalian cells, which may be either primary cells (e.g., monkey kidney), cell strains (with a finite replicating ability), or cell lines (Immortalized cells, such as Chinese hamster ovary [CHO], cells). Each has its own advantages, and bacterial, yeast, and different mammalian cells are now widely used. As a general rule, other bacterial proteins should be made in transfected bacterial cells, and human viral antigens, especially glycoproteins, in mammalian cells, because of the differences in posttranslational modification that occur in the different cell types *(30)*.

6.4. Viruses and Bacteria Used as Live Vectors

Table 3 lists viruses and bacteria being used for this purpose, as an alternative to direct transfection of cells with DNA/cDNA, and those being used (or likely to be used) in clinical trials as a vaccine. Most experience has been with vaccinia virus, because it is very easy to manipulate, has a wide host range, possesses about 100 different promoters, and as already noted to *(7)*, substantial amounts of DNA can be excised from the genome. There is room for inclusion of DNA coding for at least 10 different antigens. Unmodified vaccinia is unlikely to be used as a vector in humans because of the level of undesirable side-effects experienced during the Smallpox Eradication Program, but further modified forms are available. The avipoxes, especially canarypox, are now favored as an alternative because they undergo only an abortive infection in human cells and therefore should be completely safe. Chimeric adeno and polio viruses and Salmonella should be ideal vectors if a mucosal response is desired, e.g., by oral delivery.

Table 3
Some Viruses and Bacteria Used as Live Vectors of DNA/cDNA

Viruses	Bacteria
Animal	Salmonella[a]
Vaccinia, ORF, polio[a],	BCG[a]
herpes, varicella, adeno[b],	*E. coli*
influenza, bovine papilloma,	
Epstein-Barr, mengo, Retro	
Bird	
Fowlpox[a], canarypox[a]	
Insect	
Baculovirus[a]	

[a]Indicates those more likely to qualify soon for human use as chimeric vectors.

Making chimeric vectors has also been an effective way of assessing the potential role in immune processes of different cytokines. Inserting cDNA coding for a particular cytokine as well as that for foreign antigen(s) results in the synthesis and secretion of the cytokine at the site of infection. This approach was used to show that inclusion of cDNA coding for IL-6 greatly enhances production of s.IgA specific for antigens present in the construct *(31)*.

6.5. "Naked" DNA

The most fascinating of recent approaches and the one being pursued now most avidly is the injection of plasmids containing the DNA of interest, either directly into muscle cells or as DNA-coated microgold particles via a "gene-gun" into the skin. In the latter case, some beads are taken up by dendritic-like (Langerhans) cells and transported to the draining lymph nodes. In the case of muscle-cell injection, the possibilities under investigation are that some foreign protein expressed by the transfected muscle cells is picked up by APCs originating in the bone marrow, or that DNA escaping from muscle cells is taken up by migrating antigen-presenting cells (APCs), possibly dendritic cells. In mice at least, either approach can lead to long-lasting humoral and cell-mediated immunity (CMI) responses, including CTL formation.

Table 4
Properties and Functions of Different Components of the Immune Response

Types of response	Type of infection[a]	Cytokine profile	Stages of infectious process[b]			
			Prevent	Limit	Reduce	Clear
Nonadaptive	I		–	++	+	–
	E		–	?	?	–
Adaptive						
Antibody	I		+++	++	++	+/–
	E		+++	+++	+++	+++
$CD4^+$ Th2	I	IL-3,4,5,6,10,13				
	E	TNFα				
$CD4^+$ Th1	I	IL-2,3, IFNγ, TNFα,	–	++	++	++
	E	TNFβ	–	+++	+++	+++
$CD8^+$ CTLs	I	IL-2. IFNγ, TNFβ	–	+++	+++	+++
	E		–	–	–	–

[a]E, extracellular infection; I, intracellular infection; IFN, interferon; IL, interleukin; TNF, tumor necrosis factor.
[b]+++, very important; ++, important; +, less important; +/–, potentially important in specific cases; –, no role; ?, uncertain.

The potential of this approach is shown by two recent findings: Two chimpanzees injected frequently with plasmids containing DNA coding for several antigens of HIV were able to withstand a subsequent challenge of quite high titers of the homologous; HIV *(32)*. Second, one wk after intranasal infection of genetically susceptible mice with virulent *Mycoplasma pulmonis*, several immunizations with DNA coding for a single antigen of this organism resulted in clearance of the infection *(33)*.

This approach has numerous potential advantages. The desired plasmid should be relatively easy and inexpensive to prepare in quantity, be physically robust and heat-stable, and be effective in the presence of antibody specific to the antigen, so that repeated immunizations can be performed.

7. Properties and Functions of Different Components of the Immune Response

7.1. Classes of Lymphocytes

Our knowledge of the properties of lymphocytes, the cell type of major importance in vaccine development, has increased enormously in recent years. The major role of B-lymphocytes is the production of antibodies of different isotypes and of course, specificity. The other class of lymphocytes, the T-cells, consist of two main types. One, with the cell surface marker CD4, exists in two subclasses, the Th1 and Th2 (h standing for helper activity). A major role of Th2 cells is to "help" B-cells differentiate and replicate to form mature antibody-secreting (e.g., plasma) cells. They do this in part by the secretion of different cytokines (interleukins [ILs]) and the main ones are listed in **Table 4**. Th1 cells also have a small but important role in helping B-cells produce antibody of certain isotypes, but the overall pattern of cytokine secretion is markedly different. Such factors as interferon (IFNγ), tumor necrosis factor (TNF)α, and TNFβ have several functions, such as antiviral activity, and the upregulation of certain cellular components such as the MHC antigens of other cells, including macrophages, which can lead to their "activation." Th1 cells also mediate (via cytokine secretion) DTH, which may have a protective role in some bacterial infections. As "primary" cells (i.e., in tissues), these cells rarely have cytolytic activity, though they may gain this activity if cultured in vitro.

The other type of T-cell has the surface marker, CD8, and its pattern of cytokine secretion is similar to that of $CD4^+$ Th1 cells (**Table 4**), although they are often poor mediators of DTH *(34,35)*. As primary cells in vivo, these cells recognize and lyse cells infected by a virus or by some bacteria

and parasites, hence the name CTLs. An important aspect of this arm of the immune response is that susceptibility of the infected cell to recognition followed by lysis may occur shortly after infection and many hours before infectious progeny is produced and liberated *(36,37)*.

7.2. Recognition Patterns

Both $CD4^+$ and $CD8^+$ T-lymphocytes recognize a complex between the MHC molecule and a peptide from the foreign protein. In the former case, the peptide is derived from antigen being processed in lysosomes, whereas in the second case the peptide is derived from newly synthesized antigen in the cytoplasm. Because nearly all cell types in the body express class I MHC molecules, the role of $CD8^+$CTLs has been described as performing a continuous molecular audit of the body, searching for signs of intracellular infections *(38)*.

7.3. Role of Different Immune Responses

Table 4 ascribes particular roles to specific antibody and to the different T-cell subsets. Some general conclusions regarding adaptive responses are:

1. Only specific antibody has the potential for preventing an infection.
2. CTLs are the major mechanism for clearing most (acute) intracellular infections *(39)*; they should not be generated in an extracellular infection.
3. Antibody should clear an extracellular infection with the aid of activated cells such as activated macrophages to engulf and destroy antibody-coated particles. There are only a few rather special examples of antibody clearing an intracellular infection *(40,41)*.
4. Th-1 cells most likely contribute to the control and clearance of many intracellular infections. For example, INFγ has been shown to clear a vaccinia infection (a low-virulence pathogen for mice) in nude mice *(42)*.

7.4. The Selective Induction of Different Immune Responses

During an acute model infection (murine influenza), the sequence of appearance in the infected lung of regulatory/effector cells is, first, $CD4^+$ Th cells, then $CD8^+$ CTLs and finally ASCs. The CTLs are largely responsible for virus clearance, and it has been thought that the decline in CTL activity that occurs shortly after infectious virus can no longer be recovered from the lung *(43)*, was owing to the short half-life of these effector cells. However, it has now been found that if IL-4 formation is artificially induced very early after infection is initiated, CTL formation is substantially suppressed *(44)*. Thus, the early decline in CTL activity; which occurs as specific IgG ASCs are increasing in numbers in the lung, may be owing to two effects—a short half-life of these cells and the initiation of IL-4 production. By about 2 wk however, a pool of memory CTLs has already been formed. These persist for many months and may be quickly activated to become effector cells if a challenge infection occurs at a later time.

It is now recognized that as well as affecting the magnitude and persistence of immune responses to non-infectious preparations, adjuvants may also greatly influence the type of immune response that is induced. Adjuvants may have three roles:

1. To provide a depot of antigen;
2. To target the antigen to the plasma membrane of cells, especially APCs; and
3. To act as an immunostimulant by inducing the selective synthesis and secretion of different cytokines.

In contrast to IL-4 favoring a Th2 type response, the cytokine IL-12 favors a type 1 T-cell response and some remarkable effects have been obtained recently by inclusion of IL-12 in the antigen preparation *(45)*. The only adjuvant still registered for human use, alum, favors a Th2 T-cell response. The β-subunit of cholera toxin also favors this type of response when given orally, but it is now realized that this is owing to trace contamination with the whole toxin *(46)*. Many preparations such as water-in-oil emulsions, Freund's complete adjuvant, and ISCOMS (immunostimulating complexes containing Quil A, or fractions thereof, such as QS21), stimulate both type 1 and 2 T-cell responses *(47)*.

8. Some Factors Affecting the Ease of Development of Vaccines

Although the new technologies have made it possible in principle to develop vaccines to nearly all infectious agents, many other factors influence the ease with which vaccines can be developed and reach the market place (1; *see* **Subheading 3.2.**). Some of these factors are:

1. The simpler the agent, the more straightforward it may be to identify protective antigens;
2. If natural infection does not lead to protection, understanding the pathogenesis of infection and how the normally protective responses are evaded is helpful;
3. The occurrence of great antigenic variation/diversity in a pathogen can be a major hurdle, especially in the case of RNA viruses, because escape mutants may readily occur;
4. Integration of DNA/cDNA into the host-cell genome may lead to lifelong infection, unless the vaccine greatly limits this possibility and/or such cells are readily destroyed;
5. The availability of a readily available, relatively inexpensive animal model that is susceptible to infection and shows disease symptoms like those of humans, is a great advantage.

HIV can be quoted as an example. It suffers from disadvantages 2–5. Vaccine development, well-supported by generous funding, has been in progress for nearly 15 yr. The President of the United States has recently announced a new 10-yr plan to achieve the goal of an AIDS vaccine that will prevent the further global spread of HIV. Malaria and tuberculosis are two other diseases for which an effective and, in the latter case, a greatly improved vaccine is urgently required.

9. Promising New Developments

Despite the aforementioned constraints, there are some promising new developments.

9.1. Mixed Vaccine Formulations

Immunization of vaccinia-naive volunteers with an HIV gp160 vaccinia virus construct followed by boosting with a recombinant gp160 preparation gave higher antibody titers compared to using either preparation alone for both priming and boosting ***(49)***. An extended study has shown that even if more than 1 yr elapsed between priming and boosting, prolonged CMI responses still occurred following the boosting ***(50)***. In mice, priming with a DNA preparation followed by boosting with a chimeric poxvirus construct has given greatly enhanced specific antibody titers ***(51)***. It would be expected that this regimen would also give enhanced CTL responses. Experiments like this open up new approaches to vaccine development and it is expected that this area will undergo intense study in the next few years.

9.2. Combination Vaccines

Vaccine delivery can be a major cost component in vaccination programs. Combining vaccines so that three or more can be administered in a single shot results in considerable savings ***(26)***, so that there are determined efforts to add further vaccines to the existing combinations, DPT (diphtheria, pertussis, tetanus) and MMR (measles, mumps, rubella) (**Table 1**). To achieve this, there must be compatibility at different levels. In all cases, there is the risk of antigenic competition. This occurs at the T-cell level—competition of peptides to binding to MHC molecules and subsequent recognition by the T-cell receptor. Currently, it is not possible to readily predict the likelihood of such interference ***(52)***. However, it may be possible to minimize the risk. For example, in the case of mixtures of protein/carbohydrate conjugates, using the same carrier protein should remove the risk.

Individual components in mixtures of live viral vaccines, such as MMR, should not interfere with the "take" of other components, The use of the same vector, e.g., the same pox virus, in mixtures of chimeric constructs, should also minimize this difficulty. Again, however, the use of DNA as the basis of vaccines also offers the prospects of great advantages. Other than the possibility of antigenic competition, it is expected that combining different DNA vaccines should not be subject to this constraint.

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