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IN-DEPTH REVIEW

# Active and passive immunity, vaccine types, excipients and licensing

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**Abstract** Immunity is the state of protection against infectious disease conferred either through an immune response generated by immunization or previous infection or by other non-immunological factors. This article reviews active and passive immunity and the differences between them: it also describes the four different commercially available vaccine types (live attenuated, killed/inactivated, subunit and toxoid): it also looks at how these different vaccines generate an adaptive immune response.

**Key words** Active immunity; immunization; immunoglobulin preparations; passive immunity; vaccine excipients; vaccine licensing; vaccine types.

## Introduction

The first article of this series reviewed those host mechanisms that protect against microbial invasion. Both limited effectiveness against particular pathogens together with pathogen evasion processes mean that certain infectious diseases are still a frequent occurrence; some are occupationally related with the risk to health care workers being particularly well documented [1,2]. Since particular occupationally transmitted infections can be prevented by immunization, this article will look at how the different vaccine types modulate adaptive responses to provide further protection. First, however, the terms active and passive immunity will be considered.

## Active and passive immunity

Active immunity refers to the process of exposing the body to an antigen to generate an adaptive immune response: the response takes days/weeks to develop but may be long lasting—even lifelong. Active immunity is usually classified as natural or acquired. Wild infection for example with hepatitis A virus (HAV) and subsequent recovery gives rise to a natural active immune response usually leading to lifelong protection. In a similar manner, administration of two doses of hepatitis A vaccine generates an acquired active immune response leading to long-lasting (possibly lifelong) protection. Hepatitis A vaccine has only been licensed since the late 1980s so that follow-up studies of duration of protection are limited to <25 years—hence, the preceding caveat about duration of protection.

Passive immunity refers to the process of providing IgG antibodies to protect against infection; it gives immediate, but short-lived protection—several weeks to 3 or 4 months at most. Passive immunity is usually classified as natural or acquired. The transfer of maternal tetanus antibody (mainly IgG) across the placenta provides natural passive immunity for the newborn baby for several weeks/months until such antibody is degraded and lost. In contrast, acquired passive immunity refers to the process of obtaining serum from immune individuals, pooling this, concentrating the immunoglobulin fraction and then injecting it to protect a susceptible person.

The four most commonly used immunoglobulin preparations are as follows.

(i) Human Hepatitis B Immunoglobulin Ph.Eur.* Bio Products Laboratory: Human hepatitis B immunoglobulin is presented as two vial sizes of 200 and 500 IU. Each millilitre contains 10–100 mg/ml human protein of which at least 95% are gammaglobulins (IgG). This product is prepared from plasma from screened donors, selected from the USA. One millilitre contains not <100 IU of hepatitis B antibody. Its use occupationally is for the immediate protection of non-immune health care workers exposed to hepatitis B viruses (together with an appropriate vaccination programme).
(ii) Human Rabies Immunoglobulin Ph.Eur.* Bio Products Laboratory: Human rabies immunoglobulin is presented as a vial size of 500 IU. Each millilitre contains 40–180 mg/ml human protein of which at least 95% are gammaglobulins (IgG). This product is prepared from plasma from screened donors,

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selected from the USA. One millilitre contains not <150 IU of rabies antibody. It is given as part of post-exposure prophylaxis to non-immune individuals with a rabies prone exposure.

(iii) Human Tetanus Immunoglobulin Ph.Eur.* Bio Products Laboratory: Human tetanus immunoglobulin is presented as a vial size of 250 IU. Each millilitre contains 40–180 mg/ml human protein of which at least 95% are gammaglobulins (IgG). This product is prepared from plasma from screened donors, selected from the USA. One millilitre contains not <100 IU of tetanus antibody. It is unlikely that this preparation would be used for health care workers; it is given both as part of the management of tetanus prone wounds where there is heavy soil/manure contamination and as part of the management of all wounds if the individual is thought to be non-immune.

(iv) Human Varicella-Zoster Immunoglobulin Ph.Eur.* Bio Products Laboratory: Each vial contains 250 mg protein (40–180 mg/ml) of which at least 95% are gammaglobulins (IgG). This product is prepared from plasma from screened donors, selected from the USA. One millilitre contains not <100 IU of Varicella-Zoster antibody. It is given as part of post-exposure prophylaxis to specified non-immune individuals exposed to chickenpox.

Further detailed information about all these products is available at http://www.emc.medicines.org.uk.

## Vaccine types

The majority of workers born in the UK can be expected to have been immunized against diphtheria, tetanus, whooping cough and polio. Depending on their age and gender, they may also have had measles, mumps, rubella, *Haemophilus influenzae* type b (Hib) and *Neisseria meningitidis* type C (Men C).

These different commercially available vaccines can be classified into one of four types depending on the nature of the vaccine antigens—live attenuated, killed inactivated, toxoid and subunit. Subunit vaccines can be further subdivided into those where the antigen is produced using recombinant DNA technology and those based on normal bacteriological growth processes.

Additionally, all vaccines contain other substances (termed excipients) that are present because they improve the immune response (an adjuvant), are necessary for ensuring stability of the product (stabilizers and preservatives), are the vehicle for delivering vaccine (carrier) or are a residual of the manufacturing process (for example antibiotics or cell culture components).

### Toxoid vaccines

Certain pathogens cause disease by secreting an exotoxin: these include tetanus, diphtheria, botulism and cholera—in addition, some infections, for example pertussis, appear to be partly toxin mediated [3,4].

In tetanus, the principal toxin (termed tetanospasmin) binds to specific membrane receptors located only on presynaptic motor nerve cells. Subsequent internalization and migration of this toxin to the central nervous system blocks the metabolism of glycine which is essential for the normal functioning of gama amino butyric acid (GABA) neurons. As GABA neurons are inhibitory for motor neurons, their non-functioning results in excess activity in motor neurons with the muscles supplied by these nerves contracting more frequently than normal giving rise to muscle spasms which are a characteristic feature of tetanus.

Tetanus toxoid vaccine is manufactured by growing a highly toxigenic strain of *Clostridium tetani* in a semi-synthetic medium: bacterial growth and subsequent lysis release the toxin into the supernatant and formaldehyde treatment converts the toxin to a toxoid by altering particular amino acids and inducing minor molecular conformational changes. Ultrafiltration then removes unnecessary proteins left as a residual from the manufacturing process to produce the final product. The toxoid is physicochemically similar to the native toxin thus inducing cross-reacting antibodies but the changes induced by formaldehyde treatment render it non-toxigenic [5–7].

Following deep subcutaneous/intramuscular (sc/im) administration of tetanus vaccine, the toxoid molecules are taken up at the vaccination site by immature dendritic cells: within this cell, they are processed through the endosomal pathway (involving the phagolysosome) where they are bound to major histocompatibility complex type II (MHC II) molecules; the MHC II:toxoid complex then migrates to the cell surface. While this process is happening within the cell, the now activated mature dendritic cell migrates along lymph channels to the draining lymph node where they encounter naive T helper type 2 cells ($T_H2$), each with their own unique T-cell receptor (TCR). Identifying and then binding of the MHC II:toxoid to the specific $T_H2$ receptor then activates the naive T cell, causing it to proliferate.

Simultaneously, toxoid molecules not taken up by dendritic cells pass along lymph channels to the same draining lymph nodes where they come into contact with B cells, each with their own unique B-cell receptor (BCR). Binding to the B cell through the specific immunoglobulin receptor that recognizes tetanus toxoid results in the internalization of toxoid, processing through the endosomal pathway and presentation on the cell surface as an MHC II:toxoid complex as happens in the dendritic cell.

These two processes occur in the same part of the lymph node with the result that the B cell with the MHC II:toxoid complex on its surface now comes into contact with the activated $T_H2$ whose receptors are specific for this complex. The process, termed linked recognition, results in the $T_H2$ activating the B cell to become a plasma cell with the production initially of IgM, and

then there is an isotype switch to IgG; in addition, a subset of B cells becomes memory cells.

The above mechanism describes the adaptive immune response to a protein antigen-like tetanus toxoid; such antigens are termed T-dependent vaccines since the involvement of T helper cells is essential for the immune response generated. Polysaccharide antigens in contrast generate a somewhat different response as will be described in the section on subunit vaccines.

The rationale for tetanus vaccination is thus based on generating antibodies against the toxoid which have an enhanced ability to bind toxin compared with the toxin receptor binding sites on nerve cells; in the event of exposure to *C. tetani*, this large toxin:antibody complex is then unable to bind to the receptor so neutralizing the toxin and preventing disease development.

Diphtheria and pertussis toxoid (in acellular pertussis vaccines) are two commercially available toxoid vaccines against which antibodies are produced in an exactly analogous manner as described above. Tetanus and diphtheria vaccines (together with inactivated polio) should be offered in the occupational setting to workers who have not completed a five-dose programme. The appropriate preparation in the UK would be Revaxis which contains not $<2$ IU of purified diphtheria toxoid, not $<20$ IU of purified tetanus toxoid, 40 D antigen units of inactivated polio type 1, 8 of type 2 and 32 of type 3; the toxoids are adsorbed onto aluminium hydroxide as the adjuvant (see below).

Toxoid vaccines tend not to be highly immunogenic unless large amounts or multiple doses are used: one problem with using larger doses is that tolerance can be induced to the antigen. In order therefore to ensure that the adaptive immune response is sufficiently effective to provide long-lasting immunity, an adjuvant is included in the vaccine. For diphtheria, tetanus and acellular pertussis vaccines, an aluminium salt (either the hydroxide or phosphate) is used; this works by forming a depot at the injection site resulting in sustained release of antigen over a longer period of time, activating cells involved in the adaptive immune response. Aluminium adjuvants are also readily taken up by immature dendritic cells and facilitate antigen processing in the spleen/lymph nodes where the necessary cell–cell interactions take place that lead to the development of high-affinity clones of antibody producing B cells [9–11].

There are three principal advantages of toxoid vaccines. First, they are safe because they cannot cause the disease they prevent and there is no possibility of reversion to virulence. Second, because the vaccine antigens are not actively multiplying, they cannot spread to unimmunized individuals. Third, they are usually stable and long lasting as they are less susceptible to changes in temperature, humidity and light which can result when vaccines are used out in the community.

Toxoid vaccines have two disadvantages. First, they usually need an adjuvant and require several doses for the reasons discussed above. Second, local reactions at the vaccine site are more common—this may be due to the adjuvant or a type III (Arthus) reaction—the latter generally start as redness and induration at the injection site several hours after the vaccination and resolve usually within 48–72 h. The reaction results from excess antibody at the site complexing with toxoid molecules and activating complement by the classical pathway causing an acute local inflammatory reaction.

## Killed/inactivated vaccines

The term killed generally refers to bacterial vaccines, whereas inactivated relates to viral vaccines [3,4]. Typhoid was one of the first killed vaccines to be produced and was used among the British troops at the end of the 19th century. Polio and hepatitis A are currently the principal inactivated vaccines used in the UK—in many countries, whole cell pertussis vaccine continues to be the most widely used killed vaccine.

The adaptive immune response to a killed/inactivated vaccine is very similar to a toxoid vaccine with the exception that the antibody response generated is directed against a much broader range of antigens. Thus, following injection, the whole organism is phagocytosed by immature dendritic cells; digestion within the phagolysosome produces a number of different antigenic fragments which are presented on the cell surface as separate MHC II:antigenic fragment complexes. Within the draining lymph node, a number of $T_H2$, each with a TCR for a separate antigenic fragment, will be activated through presentation by the activated mature dendritic cell. B cells, each with a BCR for a separate antigenic fragment, will bind antigens that drain along lymph channels: the separate antigens will be internalized and presented as an MHC II:antigenic fragment; this will lead to linked recognition with the appropriate $T_H2$. Release by the $T_H2$ of IL2, IL4, IL5 and IL6 induces B-cell activation, differentiation and proliferation with subsequent isotype switch (IgM to IgG) and memory cell formation.

This process takes a minimum of 10–14 days but on subsequent exposure to the organism, a secondary response through activation of the various memory B cells is induced which leads to high levels of the different IgG molecules within 24–48 h.

Hepatitis A is an example of an inactivated vaccine that might be used by occupational health practitioners. It is a formalin inactivated, cell culture adapted, strain of HAV; vaccination generates neutralizing antibodies and protective efficacy is in excess of 90%. Vaccination should be considered for laboratory workers working with HAV and sanitation workers in contact with sewage. Additionally, staff working with children who are not toilet trained or in residential situations where hygiene standards are poor may also be offered vaccination. Primary immunization with a booster between 6 and 12 months after the first should provide a minimum 25 years protection [3].

Killed/inactivated vaccines share the same advantages as toxoid vaccines with the additional one that all the antigens associated with infection are present and will result in antibodies being produced against each of them.

Killed/inactivated vaccines have a number of disadvantages. They usually require several doses because the microbes are unable to multiply in the host and so one dose does not give a strong signal to the adaptive immune system; approaches to overcome this include the use of several doses and giving the vaccine with an adjuvant [8]. Local reactions at the vaccine site are more common—this is often due to the adjuvant. Using killed microbes for vaccines is inefficient because some of the antibodies will be produced against parts of the pathogen that play no role in causing disease. Some of the antigens contained within the vaccine, particularly proteins on the surface, may actually down-regulate the body's adaptive response—presumably, their presence is an evolutionary development that helps the pathogen overcome the body's defences. And finally, killed/inactivated vaccines do not give rise to cytotoxic T cells which can be important for stopping infections by intracellular pathogens, particularly viruses.

## Subunit vaccines

Subunit vaccines are a development of the killed vaccine approach: however, instead of generating antibodies against all the antigens in the pathogen, a particular antigen (or antigens) is used such that when the antibody produced by a B cell binds to it, infection is prevented; the key therefore to an effective subunit vaccine is to identify that particular antigen or combination of antigens [3,4]. Hepatitis B and *Haemophilus influenzae* b (Hib) are examples of subunit vaccines that use only one antigen; influenza is an example of a subunit vaccine with two antigens (haemagglutinin and neuraminidase).

The adaptive immune response to a subunit vaccine varies according to whether the vaccine antigen is a protein or a polysaccharide—subunit vaccines based on protein antigens, for example hepatitis B and influenza, are T-dependent vaccines like toxoid vaccines (as previously discussed) whereas polysaccharides generate a T-independent response.

An example of a T-independent subunit vaccine that might be administered in the occupational setting is Pneumovax made up of the capsular polysaccharide from 23 common pneumococcal serotypes which uses the capsular polysaccharide as the vaccine antigen. The vaccine is administered into the deep subcutaneous tissue or intramuscularly. At the injection site, some polysaccharide molecules are phagocytosed by immature dendritic cells (and macrophages), which subsequently migrate to the local lymph nodes where they encounter naive $T_H2$. However, the TCR only recognizes protein molecules and so even though presented by a mature dendritic cell and displayed on MHC II molecules, the $T_H2$ is not activated.

Simultaneously, non-phagocytosed polysaccharide molecules pass along lymph channels to the same draining lymph nodes where they encounter B cells, each with their own unique BCR. Because the vaccine antigen consists of linear repeats of the same high molecular weight capsular polysaccharide, it binds with high avidity to multiple receptors on a B cell with the appropriate specificity. Such multivalent binding is able to activate the B cell without the need for $T_H2$ involvement, leading to the production of IgM. Because, however, the $T_H2$ is not involved, there is only limited isotype switching so that only small amounts of IgG are produced and few memory B cells formed. In an adequately immunized individual, when *Streptococcus pneumoniae* crosses mucosal barriers, specific IgM antibody in serum will bind to the pathogen's capsular polysaccharide facilitating complement-mediated lysis. IgM is highly effective at activating complement; it is significantly less able to act as a neutralizing or opsonizing antibody.

Pneumovax should be offered to workers with chronic respiratory, heart, renal and liver disease, asplenia or hyposplenia, immunosuppression or the potential for a CSF leak: for those individuals with chronic renal disease and splenic dysfunction, where attenuation of the immune response may be expected further doses every 5 years are recommended.

T-independent vaccines can be converted to efficient T-dependent vaccines by covalently binding them (a process termed conjugation) to a protein molecule [9–11]. Following phagocytosis by immature dendritic cells, the conjugated protein and polysaccharide molecules are presented both as MHC II:protein and MHC II:polysaccharide complexes at the cell surface. Migration to the draining lymph node will bring this activated mature dendritic cell into the T-cell-rich area and lead to activation of a $T_H2$ with high specificity for the carrier protein.

Simultaneous passage of vaccine antigen along draining lymph channels to the B-cell-rich area of draining lymph nodes results in binding between the polysaccharide:protein conjugate and a B cell whose BCR has a high specificity for the polysaccharide. The polysaccharide:protein complex is internalized, phagocytosed and the protein is expressed as a cell surface complex with MHC II. There is then linked recognition between the activated $T_H2$ with high specificity for the carrier protein and this B cell. $T_H2$ involvement leads to co-stimulation and cytokine release resulting in IgM then IgG and generation of memory cells.

The advantages of subunit vaccines are the same as toxoid vaccines with the added benefit that one can distinguish vaccinated people from infected people—for example with hepatitis B vaccination, only an adaptive immune response to the surface antigen is possible whereas with infection *core* and *e* responses occur.

Subunit vaccines share the same disadvantages as toxoid vaccines, namely the need for an adjuvant (and often multiple doses), together with the frequent occurrence of local reactions at the injection site.

### Live attenuated

Variolation, a procedure developed in China and India ~1000 AD used a live smallpox vaccine to generate immunity—employing several different techniques 'well individuals' were exposed to variolous material from a human with a milder form of smallpox—presumably in the expectation that this would cause less severe disease in the recipient—an early form of 'attenuation' [3,4].

There are several approaches to attenuating a viral pathogen for use in humans. One involves growing the virus in a foreign host—for example, measles virus is cultivated in chick egg fibroblasts—viral replication in such circumstances results in the appearance of a number of mutant types: those mutants with enhanced virulence for the foreign host are then selected as potential vaccine strains since they generally show reduced virulence for the human host and this is a particularly useful approach for RNA viruses which have a high mutation rate. The molecular basis of attenuation in these circumstances is not known since the process is largely empiric and it is not possible to determine which of the observed genomic nucleotide changes are associated with diminished virulence.

An alternative approach is to grow the wild virus in an artificial growth medium at a temperature lower than that found in the human body—over time a strain may emerge which grows well at this lower temperature but multiplies so slowly in humans that adaptive immune responses are able to eliminate it before the virus is able to spread and cause infection—the cold-adapted live attenuated influenza vaccine is an example of this.

Live attenuated vaccines that might be used in the occupational setting include measles, mumps, rubella and chickenpox. Using measles as an example, the vaccine is injected deep sc/im where virions enter various cell types using receptor-mediated endocytosis. Within the cytosol, proteolytic degradation of viral proteins occurs; the peptides produced are then loaded onto major histocompatibility complex type I molecules and the complex is displayed on the cell surface. Circulating cytotoxic T cells (Tc) with the appropriate high-specificity TCRs are able to recognize the complex and release cytokines that instruct the (infected) cell to undergo programmed suicide (apoptosis) [12]. It appears that some Tc become memory cells but the basis of this is incompletely understood.

Additionally, immature dendritic cells will phagocytose virus vaccine initiating the same process previously described for protein antigens that leads to the production of plasma cells, neutralizing IgG antibodies and memory B cells.

In an adequately immunized individual, when wild measles virus is inhaled, then both mechanisms of protection work—thus for virus multiplying locally at the site of infection, Tc are able to kill infected cells; for virus that evades this and spreads through the blood stream IgG antibody there will bind it and prevent disease by neutralizing attachment to the target cell [9].

One disadvantage to live attenuated vaccines is the possibility that they may cause the illness they are designed to protect against either because they revert to virulence or because for some individuals (for example, those who are immunosuppressed) they are insufficiently attenuated.

## Conclusion

Currently available commercial vaccines are derived from live attenuated, killed/inactivated, toxoid or subunit preparations. T-independent antigens (generally polysaccharides) can be converted to effective T-dependent vaccines by conjugating the polysaccharide molecule to a carrier protein.

Varicella-Zoster and hepatitis B gammaglobulin (IgG) preparations are examples of passive immunity which have considerable applications to the occupational health situation.

## Conflicts of interest

None declared.

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