PAVENDAR BHARATHIDASAN COLLEGE OF ARTS AND SCIENCE

IMMUNOTECHNOLOGY

III B.Sc., BIOTECHNOLOGY

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Unit 5

Vaccines are biological preparations, produced from living organisms that enhance immunity against disease and either prevent (prophylactic vaccines) or, in some cases, treat disease.

Live-attenuated	Measles, Mumps, Rubella,Varicella zoster
Inactivated	Hepatitis A, Influenza, Pneumococcal polysaccharide
Recombinant sub-unit	Hepatitis B
Toxoid	Tetanus, Diphtheria
Conjugate polysaccharide-protein	Pneumococcal, meningococcal, Haemophlius influenzea type b (Hib)

Polynucleotide Vaccines:

The most recent development in vaccinology is immunization with polynucleotides. This technology has been referred to as genetic immunization or DNA immunization. The basis for this approach to immunization, is that cells can take-up plasmid DNA and express the genes within the transfected cells. Thus, the animal acts as a bioreactor to produce the vaccine. This makes the vaccine relatively inexpensive to produce. Some of the advantages of polynucleotide

immunization is that it is extremely safe, induces a broad range of immune responses (cellular and humoral responses), long-lived immunity, and, most importantly, can induce immune responses in the presence of maternal antibodies. Most recently, it has also been used for immunizing fetuses.

Thus, animals are born immune to the pathogens and at no time in the animal's life are they susceptible to these infectious agents. Although this is one of the most attractive developments in vaccinology, there is a great need to develop better delivery systems to improve the transfection efficiency in vivo.

DNA vaccines

Recently, encouraging results were reported for DNA vaccines whereby DNA coding for the foreign antigen is directly injected into the animal so that the foreign antigen is directly produced by the host cells. In theory these vaccines would be extremely safe and devoid of side effects since the foreign antigens would be directly produced by the host animal. In addition, DNA is relatively inexpensive and easier to produce than
conventional vaccines and thus this technology may one day increase the availability of vaccines to developing countries. Moreover, the time for development is relatively short which may enable timely immunization against emerging infectious diseases. In addition, DNA vaccines can theoretically result in more long-term production of an antigenic protein when introduced into a relatively nondividing tissue, such as muscle. Indeed some observers have already dubbed the new technology the "third revolution" in vaccine development—on par with Pasteur's ground-breaking work with whole organisms and the development of subunit vaccines. The first clinical trials using injections of DNA to stimulate an immune response against a foreign protein began for HIV in 1995. Four other clinical trials using DNA vaccines against influenza, herpes simplex virus, T-cell lymphoma, and an additional trial for HIV were started in 1996. The technique that is being tested in humans involves the direct injection of plasmids - loops of DNA that contain genes for proteins produced by the organism being targeted for immunity. Once injected into the host's muscle tissue, the DNA is taken up by host cells, which then start expressing the foreign protein. The protein serves as an antigen that stimulates an immune responses and protective immunological memory. Enthusiasm for DNA vaccination in humans is tempered by the fact that delivery of the DNA to cells is still not optimal, particularly in larger animals. Another concern

is the possibility, which exists with all gene therapy, that the vaccine's DNA will be integrated into host chromosomes and will turn on oncogenes or turn off tumor suppressor genes. Another potential downside is that extended immunostimulation by the foreign antigen could in theory provoke chronic inflammation or autoantibody production.

Plants Vaccine

As a new direction in the development of affordable new vaccines, transgenic plants have been developed that express surface proteins of viruses that are pathogenic to animals or humans. For example, Agricultural Genetics in England has genetically engineered the cowpea mosaic virus to include a surface antigen from the foot-and-mouth disease virus; this virus affects life stock. This genetically engineered virus was used to infect its natural host, black-eyed pea, and the introduced gene from the foot-and-mouth disease virus was expressed handsomely in the plant. The cowpea mosaic virus eventually kills the plant, and therefore the plant needs to be sacrificed a few weeks after infection. One leaf from the infected pea plant produces enough surface antigen to serve as vaccine for 200 doses.

Peptide Vaccines

The development of synthetic peptides that might be useful as vaccines depends on the identification of immunogenic sites. Several methods have been used. The best known example is foot and mouth disease, where protection was achieved by immunizing animals with a linear sequence of 20 aminoacids. Synthetic peptide vaccines would have many advantages. Their antigens are precisely defined and free from unnecessary
components which may be associated with side effects. They are stable and relatively cheap to manufacture.

Furthermore, less quality assurance is required. Changes due to natural variation of the virus can be readily accommodated, which would be a great advantage for unstable viruses such as influenza. Synthetic peptides do not readily stimulate T cells. It was generally assumed that, because of their small size, peptides would behave
like haptens and would therefore require coupling to a protein carrier which is recognized by T-cells. It is now known that synthetic peptides can be highly immunogenic in their free form

provided they contain, in addition to the B cell epitope, T- cell epitopes recognized by T-helper cells. Such T-cell epitopes can be provided by carrier protein molecules, foreign antigens. or within the synthetic peptide molecule itself. Synthetic peptides are not applicable to all viruses. This approach did not work in the case of polioviruses because the important antigenic sites were made up of 2 or more different viral capsid proteins so that it was in a concise 3-D conformation.

Advantages of defined viral antigens or peptides include:

1. Production and quality control simpler
2. No NA or other viral or external proteins, therefore less toxic.
3. Safer in cases where viruses are oncogenic or establish a persistent infection
4. Feasible even if virus cannot be cultivated.

Disadvantages:

1. May be less immunogenic than conventional inactivated whole-virus vaccines
2. Requires adjuvant
3. Requires primary course of injections followed by boosters
4. Fails to elicit CMI.

Conjugated vaccine

- Some vaccines are made by using only parts of the viruses or bacteria. These vaccines cannot cause disease, but they can stimulate the body to produce an immune response that protects against infection with the whole germ. Four of the newest vaccines are made this way.

Examples of component vaccines:

- *Haemophilus influenzae* type b (Hib) vaccine
- Hepatitis B (Hep B) vaccine
- Hepatitis A (Hep A) vaccine
- Pneumococcal conjugate vaccine

The virulence of some pathogenic bacteria depends primarily on the antiphagocytic properties of their hydrophilic polysaccharide capsule. Coating of the capsule with antibodiesd and /or complement greatly increases the ability of macrophages and neutrophils to phagocytose such pathogens. These finding provide the rationale for vaccines consisting of purified capsular polysaccharides.

Growth factors

Growth factors and cytokines are signaling molecules that control cell activities in an autocrine, paracrine or endocrine manner. They exert their biological functions by binding to specific receptors and activating associated downstream signaling pathways which in turn, regulate gene transcription in the nucleus and ultimately stimulate a biological response. A growth factor or cytokine can have various functions on different cell types while distinct growth factor or cytokines can exert similar or overlapping functions on certain cells. Growth Factors and cytokines affect a wide variety of physiological processes such as cell proliferation, differentiation, apoptosis, immunological or hematopoietic response, morphogenesis, angiogenesis, metabolism, wound healing, and maintaining tissue homeostasis in adult organisms. The abnormal production or regulation of growth factors and cytokines can cause various diseases such as cancer, liver fibrosis and bronchopulmonary dysplasia.

Growth Factor Signaling Mechanisms

- Paracrine signaling occurs between neighboring cells where the signals elicit quick responses and last only a short while due to the degradation of the paracrine ligands.

- As the name suggests, in autocrine signaling, a cell signals itself through a moiety that it synthesizes, ultimately leading to a biological response within the same cell. Autocrine signaling can either occur within the cytoplasm of the cell or, by a secreted growth factor/cytokine interacting with receptors on the surface of the same cell.

- Endocrine Signaling: In endocrine signaling, growth factor/cytokine moieties are secreted into the blood and carried by blood and tissue fluids on to the target cells whereby subsequent responses are triggered.

Cytokines

- Cytokines are a large group of proteins, peptides or glycoproteins that are secreted by specific cells of immune system.
- Cytokines are a category of signaling molecules that mediate and regulate immunity, inflammation and hematopoiesis.

- Cytokines are produced by a broad range of cells, including immune cells like macrophages, B lymphocytes, T lymphocytes and mast cells, as well as endothelial cells, fibroblasts, and various stromal cells; a given cytokine may be produced by more than one type of cell.

Classification of cytokines

- Interleukin(IL)
- Interferon (IFN)
- Tumor necrosis factor(TNF)
- Chemokines(CK)
- Colony stimulating factor(CSF)
- Transforming growth factor(TGF)

Tumor necrosis factor

- Tumor necrosis factor (TNF) is a multifunctional cytokine that plays important roles in diverse cellular events such as cell survival, proliferation, differentiation, and death.
- As a pro-inflammatory cytokine, TNF is secreted by inflammatory cells, which may be involved in inflammation-associated carcinogenesis.

Chemokines

Cytokines which recruiting monocytes, granulocytes and lymphocytes in blood to the sites of inflammation & act through G-PCR.

Types:

- CXC chemokines(α subgroup):IL-8 (Acute inflammation)
- CC chemokines(β subgroup):MCP-1, RANTES (Chronic inflammation)
- C chemokines(γ subgroup): Lymphotactin
- CX3C chemokines(δ subgroup)

Transforming growth-factor

Transforming growth-factor are cytokines which stimulate the growth of their target cells.

Include:

- Transforming growth factor-b(TGF- b)
- Epithelial growth factor(EGF)
- Vascular endothelial cell growth factor(VEGF)
- Fibroblastic growth factor(FGF)

Uses

- Interferon(IFN) in treatment of viral diseases, cancer.
- Eg. **IFN- a** treatment of condylomata acuminata (venereal or genital warts), malignant melanoma, hairy cell leukemia and hepatitis B and C, and other types of cancer including skin, kidney and bone cancers.
- **IFN-β**- multiple sclerosis
- **IFN**-Chronic granulomatous disease in conjuction with other antibacterial drugs
- Cytokines are used to enhance T-cell activation in immunodeficiency diseases, Eg. **IL- 2**, **IFN**-(Induction of Th 1 response),TNF-a
- GM-CSF & G-CSF) induces increase in white cell count
- C- To correct AIDS-associated leukopenia
- Eg. G-CSF-**Filgrastim**, used to treat neutropenia in cancer patients.