The impact of new technologies on vaccines

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ABSTRACT

Vast changes are taking place in vaccinology consequent to the introduction of new technologies. Amongst the vaccines included in the Expanded Programme of Immunization (EPI), the pertussis vaccine has been replaced by acellular purified fractions devoid of side-effects. Non-pathogenic but immunogenic mutants of tetanus and diphtheria toxins are likely to replace the toxoids. An effective vaccine against hepatitis B prepared by recombinant technology is in large-scale use. Conjugated vaccines against Haemophilus influenzae b, S. pneumoniae and meningococcus are now available, as also vaccines against mumps, rubella and measles. Combination vaccines have been devised to limit the number of injections. Vaccine delivery systems have been developed to deliver multiple doses of the vaccine at a single contact point. A genetically-engineered oral vaccine for typhoid imparts better and longer duration of immunity. Oral vaccines for cholera and other enteric infections are under clinical trials. The nose as a route for immunization is showing promise for mucosal immunity and for anti-inflammatory experimental vaccines against multiple sclerosis and insulin-dependent diabetes mellitus. The range of vaccines has expanded to include pathogens resident in the body such as Helicobacter pylori (duodenal ulcer), S. mutans (dental caries), and human papilloma virus (carcinoma of the cervix). An important progress is the recognition that DNA alone can constitute the vaccines, inducing both humoral and cell-mediated immune responses. A large number of DNA vaccines have been made and shown interesting results in experimental animals. Live recombinant vaccines against rabies and rinderpest have proven to be highly effective for controlling these infections in the field, and those for AIDS are under clinical trial. Potent adjuvants have added to the efficacy of the vaccines.

New technologies have emerged to 'humanize' mouse monoclonals by genetic engineering and express these efficiently in plants. These recombinant antibodies are opening out an era of highly specific and safe therapeutic interventions. Human recombinant antibodies would be invaluable for treating patients with terminal tetanus and rabies. Antibodies are already in use for treatment of cancer, rheumatoid arthritis and allergies. An advantage of preformed antibodies directed at a defined target and given in adequate amounts is the certainty of efficacy in every recipient, in contrast to vaccines, where the quality and quantum of immune response varies from individual to individual.

INTRODUCTION

Vaccines have had a profound effect on health care. The mere introduction of a handful of vaccines in the Expanded Programme of Immunization (EPI) drastically reduced infant mortality, especially in economically-emerging countries. Thanks to a vaccine, albeit of Jennerian descent, smallpox has been eradicated from the surface of the earth. It is expected that with aggressive and systematic use of two vaccines (the Salk killed vaccine and the Sabin oral vaccine), poliomyelitis will be eradicated globally in the near future. Similar achievements can be expected for other infections such as leprosy, where the infecting microorganism breeds primarily in humans and for which vaccines have now been developed. Vaccines not only prevent disease but also by virtue of their property of enhancing immunity in humans (and animals), render them as inhospitable territory for propagation of pathogens, thereby curtailing the focus of infection and its transmission to others.

With the input of new technologies, old vaccines are undergoing improvement. New vaccines are being made to replace those conferring insufficient or regionally variable immunity, as is the case with tuberculosis. The range of vaccines has expanded.
Infections such as hepatitis B can now be prevented by a highly effective recombinant vaccine. Vaccines are presently conceived not only against external pathogens, but also against microorganisms resident within the body, as is the case with *Helicobacter pylori* which causes duodenal ulcers or against *S. mutans* which causes dental caries.

Besides infections, vaccines are under development against cancers, control of fertility, autoimmune diseases and allergies. Advances in technologies for purification and stabilization of proteins have led to purer and safer vaccines. Genetic engineering and recombinant technology have enabled the making of antigenic components of those viruses which are difficult to cultivate *in vitro*, such as hepatitis B, hepatitis C, HIV, and human papilloma virus. Monoclonal antibodies generated by hybridoma technology are revolutionizing not only immunodiagnoses, but have also provided precious reagents for delineation of the epitopes involved in protective immunity. New methodologies for synthesis and sequencing of peptides and genes, and knowledge-based computer graphics have laid the basis of 'designer' vaccines. Last but not the least, has been the impact of better understanding of the immune system, the Th, and Th, types of immune responses and their role in conferring immunity. Parameters of non-responsive-ness or tolerance are now better understood, leading to approaches such as conjugation to carriers, to make better vaccines. New adjuvants, which have converted a non-protective vaccine against malaria to a highly protective entity are under production. Single contact-point delivery systems have been developed for administering multiple doses of the vaccines by a single shot. Oral and nasal vaccines have been shown to impart mucosal immunity, which is critically needed for some infections. Edible vaccines are on the horizon. This article aims to briefly review the fast-changing spectrum of vaccinology with the advent of new technologies. While notable trends are cited, no attempt has been made to comprehensively review individual developments in various fields.

**IMPROVEMENT OF EPI VACCINES**

The EPI proposed by World Health Organization for children has been adopted by many countries. Several hundred millions of children receive these vaccines. In the DPT vaccine, the diphtheria and tetanus toxoids are made from respective purified toxins, whereas the pertussis vaccine hitherto used in many countries is a crude entity and causes side-effects. A number of candidateacellular vaccines are under clinical trial. It is expected that in the near future, these will replace the previous pertussis vaccine. Another change expected in course of time would be the replacement of the diphtheria and tetanus toxoids by recombinant mutants of respective toxins, which retain the desirable immunogenicity but are not pathological.

* Bacillus Calmette Guerin (BCG) is still used in India and in some other countries as an EPI vaccine. Large-scale trials conducted in south India by the Indian Council of Medical Research (ICMR) with the cooperation of WHO and Centers for Disease Control of USA showed that this vaccine does not confer any protection in children against pulmonary tuberculosis. Hence, there is a pressing need to have a better vaccine for tuberculosis. We observed that two genetic strains of mice non-immunizable by BCG were protected by immunization with the *Mycobacterium W.* vaccine developed originally for immunotherapy of leprosy. Barry Bloom and his colleagues are engineering BCG with genes coding for protective antigens. In collaboration with a biotechnology company, they have obtained a mutant of BCG with diminished reaction. Similar work is being carried out by Tyagi at the University of Delhi, who has engineered mycobacterial promoters expressing homologous or heterologous genes in *M. smegmatis* and BCG. A host of secretory proteins of *M. tuberculosis* which are protective against tuberculosis in experimental animals have been identified. Their relative merits are unknown, so is the utility of employing a combination of these. What is apparent is that future vaccines for tuberculosis would be recombinant engineered propositions.

Poliomyelitis is nearing eradication. However, to achieve this goal, both the oral (Sabin) as well as the killed (Salk) intravenous vaccine would have to be employed. Research supported by WHO has indicated stabilizing modalities. The uptake of the oral vaccine is, however, not high and several repeat doses are required to induce full immunity. Also, it requires a cold chain to retain efficacy. On the other hand, the Salk killed vaccine, administered systemically, assures high immunity and has in fact been responsible for elimination of poliomyelitis in many countries.

**NEW TYPHOID AND CHOLERA VACCINES**

The previous typhoid vaccine based on killed bacilli had side-effects and imparted only short term immunity, mostly of the humoral type with no cell-mediated component. It has been replaced by two better vaccines. One of them developed by Germanier and Cryz is given orally and employs a self-limiting metabolic mutant of *S. typhi* Ty21a. It conferred very high efficacy in a trial conducted in Egypt and is commercially available. The other is an injectable vaccine developed at the National Institutes of Health, in which the polysaccharide Vi antigen is conjugated to tetanus or diphtheria toxoid to rope in the T-cell to help and enhance immunogenicity.

Holmgren et al proposed the use of the *B* subunit of cholera toxin (prepared by recombinant DNA technology) along with inactivated whole cells of *Vibrio* or *E. coli* as a vaccine. Clinical trials of the vaccine in Bangladesh showed 60%–80% efficacy. Oral live vaccines for cholera are under development in USA and India. The Indian vaccine under development seeks to employ an engineered mutant of *V. cholerae* isolated from a carrier, which is totally non-toxigenic, in which genes from a cholera toxin A chain-negative, B chain-positive *Vibrio* have been inserted. This engineered attenuated *V. cholerae* is non-reactogenic and confers high immunity against cholera in experimental models. Early clinical trials have started.

**THE FIRST RECOMBINANT VACCINE FOR WORLDWIDE USE IN HUMANS**

Hepatitis B is rampant in China and has millions of carriers all over the world. The development and introduction of a vaccine against hepatitis B was an important step forward. The vaccine is based on the surface protein of this virus, which was made in both yeast and mammalian cells by recombinant DNA techniques. The vaccine protects not only against hepatitis B but also against hepatocellular carcinoma which develops as a consequence of this infection in a certain percentage of infected people. The initial vaccines marketed by Merck USA/Pasteur–Merieux–Connaught and SmithKline Beecham were fairly expensive. Two Indian companies (Shantha Biotech and Bharat Biologicals) have obtained highly effective, efficiently expressed hepatitis B vaccines which have cut down the price substantially. More companies within India are indigenizing technology and it is expected that in the near future, this vaccine will be abundantly and cheaply available, and could be included in the EPI programme of vacci-
nation. Meanwhile, emanating from research, the second-genera-
tion hepatitis B vaccines incorporating pre S regions and naked
DNA vaccines have emerged. Michele et al. at the Institute Pasteur
have observed the safety of the hepatitis B DNA vaccine and its
ability to clear the antigen. The vaccine induces both humoral and
cytotoxic T-cell responses.9

Thanks to new technologies, the hepatitis E virus, responsible
for large epidemics has been isolated, cloned and sequenced.10 It
is transmitted by the faecal–oral route through contaminated
drinking water. A monkey experimental model of the disease has
been developed. Fortunately, this virus causes a mild self-healing
disease in adults, except in pregnant women where it can be fatal.
Significant progress has been made in delineating the molecular
structure of hepatitis C, responsible for chronic liver infection and
cirrhosis.11 Molecular tools are now available for diagnosis and
cytokines such as interferons prepared by recombinant DNA
technology are commercially available for therapy, a non-existent
possibility in the past.

LIVE RECOMBINANT VACCINES

A number of vectors have been identified and engineered to
express a variety of antigens. Vaccinia virus was an early favourite
for many reasons. It induces strong humoral and cell-mediated
responses. It has a large DNA genome, in which 25 kb of foreign
DNA can be inserted at the TK locus.12 The virus does not get
integrated in the host genome and multiplies in the cytoplasm of
the host. The genes of hepatitis B, Herpes and many other viruses
have been engineered in vaccinia. However, those which have
reached field-use and commercialization stages are the vaccinia-
based live recombinant vaccines for animals. Vaccinia–rabies
glycoprotein imparts high efficacy against rabies in dogs and
other mammals tested.13 The vaccine can be used intradermally or
through a bait, which on chewing transfers the engineered vacci-
cinia on to the oral mucosa. Large-scale use of this vaccine in
Europe and USA has enabled a drastic reduction of rabies in wild
foxes and raccoons. Another remarkable success has been in the
control of rinderpest in Africa by the use of a recombinant vaccinia
expressing two proteins of the rinderpest virus.14,15

At times, it is important to anchor the vaccinia-expressed
protein on the membrane of the host cell to induce good immune
response. This was the case when the β subunit of hCG (human
chorionic gonadotrophin) was engineered in vaccinia. A low
antibody response was observed with β-hCG alone. However, a
high antibody response was observed (every animal exhibiting
high titres of antibodies against hCG) when β-hCG was co-
expressed with a 48 amino acid-fused peptide anchored in the
membrane.16 The vaccine engendered a long-duration, very high
antibody response in monkeys during which they were protected
from pregnancy.17

Canary pox virus has replaced vaccinia for human vaccines, as
the avian pox viruses express the engineered antigen but do not
replicate in humans, hence these are deemed to be safer vectors.
Clements-Mann et al. have made a vaccine against HIV18 in this
vector, which has entered Phase II and Phase III trials. We have
expressed β-hCG–rabies glycoprotein (as membrane anchor) in
fowl pox virus, which expresses β-hCG on the surface of the host
cells.19

Two other interesting vectors being experimented with for live
recombinant vaccines are avirulent Salmonella, and adenoviruses.
The former has the advantage that it can be used orally, the latter
appears to be carried widely by humans without any apparent ill
effects. Curtiss et al.20 have developed genetic strains of Salmon-
ella, which are safe and in which the engineered genes can be
expressed to elicit high humoral and mucosal response. A sperm-
specific protein SP-10 expressed in a recombinant Salmonella
is undergoing investigation to determine its fertility-controlling
potential. The hepatitis B surface protein has also been expressed
in this avirulent vector.

NUCLEIC ACID VACCINES

The observation that naked DNA introduced in either skin or
muscles generates both cell-mediated immunity and antibody
response against the protein encoded by administered DNA was
a major advance.21 Plasmids with eukaryotic promoter, enhancing
oligonucleotide motifs, eukaryotic termination and poly A sig-
nals22 are available commercially to enable engineering of the
desired DNA. Engineered plasmids can be replicated in a fast
growing bacteria, for instance E. coli, to obtain large amounts of
DNA vaccine at low cost.

DNA vaccines have been made against two hypervariable
viruses, HIV and influenza. The testing of one of the HIV-1 DNA
vaccines was done in cynomolgous monkeys and the feedback of
immunization was favourable, as the vaccine could produce broad
immune reactivities against several regions of the envelope pro-
tein of the virus (e.g. gp120, gp41) and provided additive protec-
tion by stimulating both T-helper cell and cytotoxic T-lymphocyte
(CTL) response against HIV-1 antigens.23 The antibodies which
were neutralizing the virus were measurable within 30 days of
inoculation.

Experimental studies on influenza DNA vaccine were carried
out in mice and immunization led to generation of specific CTL
response and protection from a subsequent challenge with a
heterologous strain of influenza A virus.24 HIV and influenza
virus mutate rapidly and have hypervariable regions. In both
cases, it was of great interest that the immune response was
generated against both variable and constant domains of the
antigen, with the result that the immune response would prevail
over minor variations.

DNA vaccines have now been made against a variety of other
infections. Of particular interest are those against hepatitis B,25
rabies,26 tuberculosis,27 and malaria.28,29 Both malaria and tubercu-
losis would demand immunization against more than one antigen
for high protective efficacy. The malarial parasite passes through
multiple stages—sporozoite, merozoite and gamete—each hav-
ing vulnerable critical antigens. An advantage offered by DNA
vaccines is the possibility of combining genes corresponding to
several antigens in the same construct. Another benefit of nucleic
acid immunization is simultaneous induction of both cytotoxic T-
cells and neutralizing antibodies. This happens by processing and
presentation of antigens coded by DNA of both class I and class
II MHC molecules.

A number of concerns were raised about the safety of DNA
vaccines. For some of these, salient observations have been made.
Unlike live viral vaccines, DNA vaccines have no potential of
causing infection. No case of integration of the DNA vaccine has
been noted in the chromosomes of the host cells, nor has any
malignant transformation or induction of autoimmune response
occurred. At present, these vaccines appear to be safe and devoid
of toxicity and side-effects. An in-built advantage of DNA vac-
cines is the possibility of testing them in various mammals and
experimental laboratory animals, as the plasmid carrying the
DNA infects all eukaryotic cells.

Currently, clinical trials using DNA vaccines are underway for
herpes, influenza, hepatitis B, HIV and malaria. A DNA vaccine
for HIV-1 env and rev genes has been tested for safety and host immune response in 15 HIV-infected asymptomatic patients receiving no antiviral therapy. Patients received three doses of the vaccine at 10-week intervals in a dose-escalation trial. Data were analysed for CTL responses, T-lymphocyte proliferation and \( \beta \)-chemokines. More changes were noted in these immunological parameters in the high-dose groups, thus showing a stronger anti-HIV immune response. The vaccine was well tolerated and no side-effects ascribable to immunization were noted.

DNA vaccines exhibit few limitations and many positive points. These can be used to raise immune responses against the protein components of the pathogen. However, certain microbes have outer capsular structures of polysaccharides, which carry the protective antigens. DNA vaccines cannot substitute for the polysaccharide-based vaccines.

**MINIMIZATION OF VISITS FOR VACCINATION**

The full course of childhood immunizations currently requires up to 18 visits to a clinic. This is not only costly, but failure of compliance for repeat injections leaves a percentage of children incompletely immunized. Attempts are being made to develop combination vaccines. For instance, a quadruple vaccine combines polio with DPT. Similarly, hepatitis B and *Haemophilus influenzae* vaccines are being tested as partners in combination vaccines.

Two alternate strategies are also possible. In one, multivalent DNA vaccines are being conceived to carry protective proteins coding genes for more than one infection. The second strategy is to develop biodegradable delivery systems, whereby multiple doses of the vaccine can be given at a single contact point.

**VACCINE DELIVERY SYSTEMS**

Over 70 years ago, Raman discovered that the antitoxin response to tetanus and diphtheria toxin was increased by injection of vaccines together with other compounds such as starch, oil, saponins or even breadcrumbs. Glenny *et al.* reported the immune stimulating properties of aluminum salts. Vaccine technology has come a long way since then and so has the technology for the delivery of antigens.

New delivery systems and adjuvants are being investigated that would potentiate the immunogenicity of antigens which otherwise induce 'weak' immune response when given adsorbed on alum. The inclusion of vaccines in liposomes or biodegradable microspheres has been found to be useful for developing single-contact point delivery systems.

**Liposomes**

Liposomes have been successfully used as drug carriers and tested as carriers for antigens. In 1974, Allison and Gregoriadis identified these as potent, non-toxic adjuvants that enhance immune response. These are microspherical structures consisting of vesicles that are concentric bilayers or multilayers of phospholipids and cholesterol in which antigens can be introduced in aqueous or lipid phase. In most cases, the association of the antigen with the lipid vesicle has been by their internal entrapment in the aqueous phase. The adjuvant property of liposomes is due to their depot effect and the ability to target the encapsulated antigens to antigen-presenting cells (APCs) because of their greater susceptibility to phagocytosis. They induce both humoral and cell-mediated immunity.

Additional adjuvants can also be incorporated into liposomes together with the antigen. Incorporation of Lipid A has been shown to markedly improve the immune enhancement capacity of liposomes.

One non-phospholipid liposomal preparation (Novasome vesicle) available commercially for two poultry vaccines is approved by the US Department of Agriculture. Recently, another liposome-based preparation, namely Epaxal Berna, against hepatitis A has also been licensed in Switzerland.

**Biodegradable microspheres**

The development of controlled release formulations for vaccine delivery was a priority area of research for the WHO. Microspheres made of biodegradable polymers slowly release the encapsulated antigen into the tissue of a vaccinated individual, while simultaneously serving as a repository for unreleased antigen, a phenomenon known as the depot adjuvant action. The subsequently released antigen acts as a secondary stimulus to the sensitizing action of the antigen released earlier, leading to sustained antibody production.

One of the first studies demonstrating the concept of a single-shot immunization was the work of Preis and Langer in 1979 using a non-degradable polymer, polyethylene-vinyl acetate. A major disadvantage of non-biodegradable polymers is that these must be retrieved surgically after the expiry of the delivered antigen. The long term effects of these polymers have not been determined and these can have potential adverse side-effects as have been observed for silicone breast implants, previously considered safe and biocompatible.

Biodegradable polyesters based on lactic and glycolic acids are approved for human use. They have been employed for the last 20 years in resorbable sutures and have a well-established safety and toxicology record. Polycaprolactone, an inexpensive alternate to PLGA polymers, is also approved for human use.

Preclinical investigations in experimental animals have yielded promising results and the use of microspheres as delivery systems for vaccines appears possible in the near future.

A subunit vaccine for HIV-1 consisting of recombinant glycoprotein 120 (rgp 120) when given in microspheres together with QS21 adjuvant, elicited long term bioneutralizing antibody titres in baboons. A single immunization with tetanus toxoid entrapped in microspheres generates protective antitoxin antibodies in mice which are comparable to those elicited after immunization with the conventional alum adsorbed vaccine. Our laboratory has shown that luteinizing hormone releasing hormone (LHRH) conjugate, an anti-prostatic cancer vaccine, encapsulated in PLGA microspheres given along with alum and SLP/PS adjuvant in rodents, not only generates a sustained antibody response for 5 to 7 months but the bioeffective antibody response is initiated within 2 weeks post immunization. The induction of a similar response takes 8 weeks when the vaccine is given adsorbed on alum after three immunizations.

Microspheres are taken from the intestine by Peyser's patches, hence have considerable potential as carriers for oral and nasal immunization. Microencapsulated staphylococcal enterotoxin B or ricin toxoid when given orally or nasally in mice have been shown to induce not only mucosal IgA response but also systemic immune response. Holmgren *et al.* observed the prevention of allergic encephalitis if myelin protein is given orally. The nasal and oral routes are advocated for anti-inflammatory vaccines for multiple sclerosis and insulin-dependent diabetes mellitus.
IMMUNE STIMULATING COMPLEXES (ISCOMs)

ISCOMs have a micellar structure which consist of glycosides of the adjuvant Quil A, cholesterol, the antigen, and, in most cases, phospholipids. ISCOMs have been proven to be potent stimulants of both humoral and cell-mediated immune responses. It has been suggested that these complexes act as stimulants by targeting antigens to APCs. ISCOMs are used in veterinary vaccines and have not been approved so far for human vaccines. Attention is being paid to the reduction of toxicity of the respective components of ISCOM preparations, especially Quil A. Ultimately, the ISCOM approach may generate promising vaccines against viral infections.

HUMANIZED AND HUMAN RECOMBINANT THERAPEUTIC ANTIBODIES

With the emergence of new technologies, a new mode of immunotherapy has dawned, which offers the use of preformed human or chimeric antibodies of desired specificity and characteristics. These can be administered in the appropriate dose to achieve efficacy in every recipient, doing away with the uncertainty and quantum of antibody response to a vaccine, which varies from individual to individual. Antibodies as ‘drugs’ would be highly safe in situations where the target molecule is defined. The in-built specificity of the antibody for the given target molecule would avoid side-effects invariably associated with pharmacological preparations.

Monoclonal antibodies (MoAb) have been used in therapeutic clinical trials as: (i) mediators of immune effector function; (ii) carriers of cytotoxic agents (magic bullets); (iii) agents to block tumour growth factor; or (iv) anti-idiotypic vaccines.

Anti-idiotypic MoAb can mimic both protein and non-protein antigenic epitopes. In animal models, and now in humans, it is possible to induce immune responses against tumour antigens using anti-idiotypic MoAb vaccines. They have potential advantages when the antigen is not readily available in sufficient quantities or purity, or when the antigen is a non-protein moiety. Yao et al. used BEC2, an anti-idiotypic mouse MoAb that mimics GD3 ganglioside for immunization of melanoma patients. Clinical trials demonstrated that BEC2 with BCG can induce anti-GD3 antibodies in patients. Zhang et al. have developed an MoAb against GD2 (mAb 3F8), richly expressed at the cell surface of human neuroblastomas, sarcomas and melanomas. In a syngeneic murine model, they demonstrated that passively administered and vaccine-induced anti-ganglioside antibodies prevent outgrowth of micrometastases. The level of protection was proportional to the antibody titre. Also, protection was demonstrated even when immunization was initiated after tumour implantation.

Monoclonal antibodies against tumour-associated antigens have also been tested with various imaging techniques to improve detection and staging of lung cancer. Geneticall-engineered chimeric human–mouse MoAbs have been developed by replacing the mouse Fc region with the human constant region. The starting material is the mouse hybrid cell clone, making MoAbs of high affinity and proven bioefficacy. The RNA of these cells is reverse transcribed employing PCR and primers coding for the variable heavy and light chains. These are cloned, amplified and sequenced to confirm that the putative CDRs have framework residues of immunoglobulins. The variable light and heavy chains are then linked into a single chain variable fragment (ScFv) with a spacer in between, enabling the flexibility of conformation of the two variable chains to cooperate for binding with the target antigen. The ScFv is then expressed in a prokaryote host to determine whether the recombinant product has the requisite specificity and affinity for binding. Chimeric antibodies can be obtained by retaining the mouse antibody-binding regions and aligning these with constant regions of the human immunoglobulin genes. Alternatively, ScFv is used as a probe for sifting complementary human variable regions from a library to eventually make a totally human antibody. Site-directed mutagenesis may be necessary to achieve high-affinity human antibodies. Antibody gene constructs can be expressed in yeast or in plants. Gene expression in plants has several advantages. An antibody expressed in plants would not demand exclusion of oncogenic DNA, endotoxins and eukaryote pathogens during the purification procedure. High yields of antibodies are obtained and they can be stored in a stable condition in seeds. Plants are able to make antibodies efficiently. Even a complex antibody such as IgA has been made successfully by Ma et al. in plant cells. This antibody directed at S. mutans protects against dental caries. The antibody stays in the oral cavity for three days after a single rinse, in contrast to IgG which stays only for 24 hours.

A humanized antibody against the FeRl binding regions on IgE is undergoing clinical trials in Switzerland. Akatsu et al. in Japan are planning treatment with anti-IL-5 antibody. Ozakiet al. have reported that humanized anti-HM1.24 MoAb that detects human plasma cell-specific antigen has potential as a new therapeutic agent in multiple myeloma; and that co-treatment of effector cells with immunomodulating cytokines, interleukin-2 (IL-2), IL-12 or IL-15, restores the effect of humanized anti-HM1.24 MoAb in patients with diminished antibody-dependent cell-mediated cytotoxicity (ADCC) activity.

Another major trial with a chimeric anti-TNFα antibody has been carried out by Feldman et al. in rheumatoid arthritis. A dose of 3 or 10 mg/kg body weight was given for 12 weeks. Seventy per cent of patients experienced improvement and a few achieved clinical remission.

Monoclonal antibodies of mouse origin have been used for some years for therapy of tumours in humans. Their replacement by human or chimeric antibodies would avoid anti-mouse sensitization on repeat administration. With human, mouse or chimeric (human–mouse) MoAbs, beneficial results have been obtained for treatment of colorectal cancers and refractory neuroblastoma and osteosarcoma. The antibodies exercise their effect by complement-mediated lysis and by ADCC. These effects are augmented by co-therapy with recombinant cytokines IL-2, GM-CSF and CSF. A single chain human ScFv co-expressed in alignment with IgG, against the melanoma-associated chondroitin sulphate proteoglycan causes specific lysis of human melanoma cells by NK cells and complement mediation.

Antibodies with two binding ends—bispesific antibodies (BsAb) provide improvement for targeting cancer cells. These are prepared by chemically linking two different MoAbs or by fusing two hybridoma cell lines to produce a hybrid—hybridoma. BsAbs have been used to demonstrate that specific surface molecules can trigger leucocytes to either phagocytose or kill tumour cells, viruses, parasites and infected cells. They have also been used to direct toxins to tumour sites and fibrinolytic agents to areas of thrombosis.
An important group of antigens encoded by the melanoma antigen encoding gene (MAGE) family of genes is expressed in a significant proportion of melanoma tumours of several histological types. These genes are not expressed in normal tissues except the testis. Clinical trials are underway to immunize patients suffering from metastatic melanoma using peptides coded by MAGE. Regression has been observed in 5 of the 17 immunized patients.

A novel way to enhance the immunogenicity of tumour-associated antigens is by transfection in dendritic cells, which process and present the antigens to induce both cytotoxic and humoral antibody responses. Fonget et al. have designed a vaccine for prostate cancer whereby they primed the human dendritic cells with xenogenic prostatic acid phosphatase (rat PAP) and administered it to patients with prostate cancer. The assessment of the immune response of patients after 12 weeks of the initial dose of mouse PAP-pulsed dendritic cells indicated a stability in the levels of prostate specific antigen (PSA), a marker of prostate tumour activity.

Besides immunization against tumour-associated antigens, another approach for vaccine development is to immunize against a key growth factor supporting the proliferation of cancer cells. Pilot clinical trials are underway to define the safety, toxicity and immunogenicity of active immunotherapy with human epidermal growth factor (huEGF) coupled to a carrier protein. Patients in advanced clinical stages of malignant carcinomas (colon, lung, stomach and prostate) were immunized with huEGF linked to either tetanus toxoid (TT) or Neisseria meningitides recombinant protein (P64k) inducing anti-EGF antibody titres without evidence of toxicity.

Prostatic hypertrophy and carcinoma may also be amenable to control by administering vaccines and relevant MoAbs. Talwar et al. developed a semisynthetic vaccine against LHRH, in which D-glycine at position 6 was substituted by D-lysine to freeze the native conformation of the decapetide, concomitantly creating a functional group to link the decapetide to a spacer and a carrier. The vaccine causes marked atrophy of the rat and monkey prostate. The vaccine also inhibits the growth of Dunning tumours in rats. After preclinical toxicology, drug regulatory and ethical approvals, the vaccine has been clinically tested in 28 patients suffering from D2 advanced prostatic carcinoma in India and Austria. In all patients, the vaccine was well tolerated, and in those generating 400 pg/ml or more of antibody titres, clinical benefits were observed. The levels of PSA and PAP declined sharply in these patients. Serial nephrostograms and ultrasound scans indicated the regression of tumours. Development of a recombinant vaccine and humanized anti-LHRH antibodies is ongoing.

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