IMMUNO-BIOTECHNOLOGY—SCOPE AND PERSPECTIVES*

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A remarkable property of the living systems is to react, to overcome infection and to survive in spite of a plethora of hostile organisms. This phenomenon is *sine qua non* to life and its preservation. A constant battle goes on between the parasite and its host, the survival of the former is dependent on its escape from the curbing influence of the latter. Immunology is an exciting area of contemporary research in life sciences. The growing knowledge about the mechanisms by which this apparatus performs its functions, is being increasingly harnessed to formulate new products for therapeutic and diagnostic purposes.

The immune system operates through cells and molecules. A normal human body has an average of 10^{12} cells and about 10^{20} molecules. The cells are atleast of three types with subtypes. They have the capacity to engulf the invading organism and kill it by a conjoint interplay. Interaction of these cells elicits the formation of the protective molecules, amongst which are the antibodies. Antibodies have the ability to recognize and discriminate between molecules. This property of specific recognition is made use of in diagnostic tests. By binding with the substance against which they are elaborated, which may be present on a virus, bacteria or toxin, they inactivate the harmful agent and facilitate its disposal by scavenging mechanisms. This is the rationale in the use of sera containing performed antibodies for treatment of acute cases of diptheria, tetanus and snake bite or in taking a shot of globulins as a prophylactic cover for hepatitis, measles, etc. An important technological advance has been made in recent years to develop an approach which permits the production of nearly pure and homogeneous antibodies by fusion of an antibody forming cell with a cell capable of perpetual multiplication. This is opening out new possibilities for diagnostics and therapy. Another important development is taking place, which is likely to usher in a totally new generation of vaccines.

I. ACTIVE IMMUNIZATION APPROACH—THE VACCINES

It has long been known that in a community, every one does not suffer from a disease in spite of exposure to infection. Those who become victims, represent the fraction where the tussle between the load of infectious agent overweighs the potency of the immune response. Thus to keep healthy there are two ways: (i) to avoid infection or minimize infectious load and (ii) to invigorate immunity. The former requires better standards of hygiene, sanitation and environmental control, often dependent on socio-economic conditions. The latter, the vigour of the immune system is generally influenced by factors such as good nutrition, but is amenable to specific potentiation by deliberate intervention. This is what the vaccines seek to achieve. Vaccines have been the most important agents responsible for control of epidemics and containment of the diseases. Calamities such as plague described so vividly by Albert Camus in one of his novels around the turn of the century are seldom heard of today, thanks to the vaccine. The incidence of polio causing serious deformities was relatively high in countries where the standard of living and hygiene are better (USA, Scandinavia). It dropped off only after the introduction of a vaccine in the mid-fifties. Tetanus, whooping cough, and diptheria are rare in countries where immuno-phophylaxis or vaccination is widely practiced. The world-wide eradication of the dreaded disease—small pox—may never have been possible without a vaccine. Vaccines are cost-effective and redress the health problems posed at the community level. They

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operate by mobilization of a natural indigenous capability. They require periodic intake. They do not demand highly trained manpower, and can be administered by para-medical auxillary health personnel.

New developments in the Vaccine field

(1) Changes have taken place in the technology for production of vaccines. Notable advances have been made in the cultivation of virus in tissue culture, for which special cell lines have been developed and processes refined to produce large batches. This would have an impact on production practices, resulting in supply of better vaccines for rabies, measles, polio, etc in the developing countries in line with the rest of the world.
(2) Vaccines against several diseases were introduced after limited trials. Frequently these were conducted in a given location and little heed was paid to the possibility that a vaccine workable in one country may not be effective in another environment. Over the years trials with BCG in different countries have indicated wide variations in the degree of protection conferred by this vaccine to pulmonary tuberculosis. The consideration of environmental factors is thus important in drawing conclusions on the efficacy of a vaccine. Experience with polio vaccine has another take-home lesson. The first vaccine introduced was the killed vaccine elaborated by Jonas Salk. It brought down the incidence of polio cases quite sharply in USA and elsewhere where it was employed. Subsequently due to a mishap in a production batch in which full killing of the virus was not achieved, vaccination produced cases of polio. The vaccine was withdrawn by the Drug Regulatory Agency in USA and instead, the oral attenuated vaccine developed by Sabin, introduced. Several other countries like Canada, Sweden, Holland, however, continued with the Salk Vaccine. In India the oral Sabin vaccine was adopted without proper control trials, as also in many other countries of Africa and Asia. Recent investigations in India and some African countries indicate that the oral attenuated vaccine is not taken up by a fairly high percentage of recepients. On the other hand, the killed polio vaccine of Salk imparts a better immunity. It is thus not inconceivable that there will be a change in the type of polio vaccine to be used in this country.
(3) The old cholera vaccine has been given up for lack of good enough efficacy. New vaccines are under development. One of them is the subunit of the cholera toxin, which by itself is not toxic to the gut cells but can evoke the formation of antibodies neutralizing the bacterial toxin. A mutant strain with self-limiting replication characteristics has also been developed, but requires clinical testing.
(4) The old typhoid vaccine is of limited value with short duration of immunity. It does not induce cell-mediated immunity and has thus no protection against relapse. A new oral vaccine based on a mutant strain was developed by Germanier in Switzerland. Clinical trials in Egypt have given encouraging results.
(5) New vaccines are at an advanced stage of development against bacterial polysaccharides. An example is the vaccine against the Hemophilus influenzae developed by John Robbins and Collaborators[1].
(6) Another recently introduced vaccine is against the hepatitis-B. It utilizes the viral coat protein prepared from the blood of donors who are "carriers" of the virus. The vaccine is in clinical use, even though fairly expensive. The second generation of the vaccine is, however, already in sight. The coat protein has been cloned by DNA recombinant technology and expressed in both *E. coli* and yeast by the Institute Pasteur Group led by Pierre Tiollais[2] and others. Another interesting and concurrent development in the field is the delineation of the primary structure of this protein which consists of 226 amino acids. A number of research laboratories are working on the hot track of identifying the segment(s) of this protein which have the capability of engendering antibodies inactivating the virus. Progress is rapid and the propositions made by different investigators highlight the importance of a region within the polypeptide chain where the essential information for the immune system is resident (table 1). Another interesting approach is the insertion of the

hepatitis-B surface protein gene in vaccinia by Bernie Moss and colleagues at the NIH. The gene is expressed in the host cell. This vaccine produces a fairly good antibody response.

Synthetic Vaccines

A new era is beginning in vaccinology. Since the introduction of the first lot of vaccines using formalinized or heat-killed organisms, attempts have been made to purify the important constituents and use only these instead of the total organisms. By this approach, undue toxicity due to irrelevant compounds can be avoided. This process has gone further. It is realized that even in a pure protein molecule, there are regions which stimulate the immune response, others may be suppressive or nonconsequential for the immune system. This knowledge is giving rise to the concept of submolecular vaccines. Why not map the determinants which engender the right type of response and do away with the rest of the chain? This is indeed what is happening at a very rapid pace. The base sequence of a gene can be determined very fast by the elegant methods perfected in recent years. The gene base sequence can be written down in terms of a protein sequence by the known principles of the genetic code. The next question that poses is on the definition of the subpart of the molecule, which is vital for the immunological response. The approach initially adopted was to cut the protein into several fragments by the action of enzymes and or synthesize chemically several parts of the molecule and sift out those which bind with the antibodies generated by the whole molecule. This laborious method is now being complemented by another elegant approach—the computer graphics. With the coordinates already worked out, the computer projects the three-dimensional structure of the protein. Various facets are examined and particular attention given to the disposition of the polar amino acids in the overall structure. It is rightly surmised that these will be the residues looking outwards and would be in contact with the aqueous environment. To be further sure, through a computer program, a molecule of water is schematically rolled over the outlying interfaces to demarcate the points where water can fit in. These are believed to constitute the epitopes on the surface of the molecule. These would be the determinants contacted and recognised by the lymphocytes to generate a response. This approach has been utilized successfully by Richard Lerner and a team of imaginative competent scientists at the Scripps Institute, La Jolla. The result of their work is a growing list of peptide sequences for key viral proteins which may replace the traditional vaccines (table 1). Similar work has proceeded in many other laboratories, complementing, reinforcing or correcting the so-called core sequences. These may be continuous determinants in the sequence of a protein or conformational determinants, where two distant parts of the chain come close together at a point linked by S–S bonds and create a shape recognized by the immune cells.

The story of foot and mouth disease (FMD) is illustrative of the trend and the nature of the development that is on. The disease is caused by a Picornavirus. About 35 species of animals including cattle, sheep, swine, goats fall victim to this infection. The mortality is high in young animals, in adults there are severe deformities. The virus was isolated, grown in cells of baby hamster kidney or bovine tongue epithelium, and a vaccine based on killed virus introduced several

Table 1 *Identified Epitopes for some Synthetic vaccines*

	Amino acid Residues	Reference No.
1. Foot and Mouth Disease	20 (141–160)	(5)
2. Hepatitis	34 (48–81)	(43)
	12 (138–149)	(44)
	16 (122–137)	(45)
3. Diphtheria	16 (186–201)	(46)
4. Streptococcus Pyogenes Infection	CB 7–35 (Repeating Units)	(47)
	12 (S 18–29) CB 7	(47)

years back. The virus coat is composed of 240 protein molecules, 60 copies each of the four proteins VP_1, VP_2, VP_3, and VP_4. Attempts were made and indeed success achieved in cloning a chimeric molecule consisting of a protein of the Trp-promoter-operator system with VP_3. This chimeric protein when injected into infected animals elicited protective immunity[3]. This achievement theoretically does away with the total virus vaccine and all the hazards associated with it. Virus can escape complete inactivation in a production batch with dire consequences. The moment it is based on a protein produced independently of the virus no such risk is involved. Synthetic vaccines or those based on proteins cloned by DNA recombinant technology would therefore be more safe as far as these hazards go. They would also be more stable.

The third step in FMD vaccine development has also been taken. Lerner and his colleagues[4] have deciphered a 20 amino acid piece of the VP_1 protein (table 2). This has also been synthesized chemically[5] and the peptide when conjugated to a carrier elicits antibodies in rabbits neutralizing the virus.

Table 1 summarizes the sequences of epitopes in some of the pathogens which have been identified and which have the potential of constituting the synthetic vaccines. In some cases, the small peptide has better immunogenicity than the total protein. The list is growing. It is believed that a small peptide sequence has very recently been delineated in malaria parasite by Ruth Nussenzweig. The sequences for polio, rabies are in the pipeline. Once delineated, these can be made chemically in unlimited amounts, or produced by DNA recombinant technology.

Table 2

(a) Foot and mouth disease: VP_1

141
H-Val-Pro-Asn-Leu-Arg-Gly-Asp-Leu-Gln-Val-Leu-Ala-Gln-
160
OH-Pro-Leu-Thr-Arg-Ala-Val-Lys

(b) Diphtheria toxin

H-Cys-Ala-Gly-Asn-Arg-Val-Arg-Arg-Ser-
201
Val-Gly-Ser-Ser-HO-Cys-Lys-Leu

Another interesting and important development is the building of an adjuvant moiety along with the core peptide sequence. The Pasteur group of Chedid *et al* together with Michael Sela and Ruth Arnon of Weizmann Institute, Rehovoth have come out with vaccines for diptheria[6] and LHRH[7] by linking the peptide with the muramyl-dipeptide. The conjugation reduces the pyrogenicity of MDP and apparently gives rise to a highly immunogenic and biologically potent preparation.

Structured and non-conventional vaccines

Vaccines were developed in the past primarily against infectious organisms. More recently a new breed of vaccines are being developed. They differ from the conventional vaccines in atleast two respects. They are directed against endogenous, rather than exogenous proteins; furthermore they are structured in design. Examples are the anti-fertility vaccines, which mobilize the body's immune system to react against chosen hormones and proteins critical to the success of human reproduction. Contraceptive vaccines are conceptually new approaches. The first anti-fertility vaccine to come up for Phase-I clinical pharmacology trials conducted in five different countries was the anti-hCG vaccine[8]. It was composed of a subunit of the pregnancy hormone β-hCG linked chemically to a bacterial protein, the tetanus toxoid. The conjugation with the carrier enabled the overcoming of the immunological tolerance to a "self" protein and the recepients made antibodies against both hCG and tetanus toxoid. The design of the vaccine was such as to bring about protection against a health hazard, while at the same time, antibodies were generated against the pregnancy hormone. The antibodies were competent in the neutralization of biological activity of the hormone *in vivo* and *in vitro*[9,10]. The antibodies generated by the vaccine were effective in baboons for termination of early pregnancy[11]. The primates tested at the experimental stage and the women immunized in

phase-I clinical trials continued to ovulate normally. No disturbance was seen in endocrine, metabolic and other functions[12]. Immunization was reversible and the antibody titres declined to near-zero levels in every case in course of time. Phase-I studies demonstrated the procedure to be free of side effects[13–16]. The main limitation of the first prototype of the vaccine was the large variability in titres from individual to individual. Although such variability should have been expected, and is also observed with other proteinic vaccines, it may not be a desirable trait for the anti-fertility vaccines, as recipients with low titres will be prone to pregnancy. There is thus need to improve the formulation by addition of an adjuvant. Research during the last couple of years has identified some compounds with immunopotentiating properties. We have also considered the use of polyvalent antigens, so as to overcome the constitutional variations of immune response amongst individuals. In the last few years our research has extended to include three other vaccines. One of them is against the β-subunit of ovine-LH, the other against a key decapeptide hormone LHRH, which regulates both male and female fertility and is thus usable in both sexes. The third vaccine is against the glycoproteins present in the zona layer surrounding the mammalian egg[17]. Antibodies against these proteins impose a pre-fertilization block.

Immunological approaches to control fertility were received with scepticism in mid-seventies when they were first formally proposed. The area has, however, grown and a large number of laboratories in many countries of the world are engaged in study of the various facets of basic and applied reproductive immunology. Two international journals solely devoted to immunology of reproduction are now published. Immunology of reproduction figured as an official theme of symposia and plenary lectures in the International Congress of Immunology, Endocrinology, Fertility and Sterility and Human Reproduction held in the last 3 years. The second Congress of Immunology of Reproduction was scheduled to meet in Kyoto, Japan in 1983. The field is thus strengthening and expanding at a rapid pace. Aquisitions of research in this area will be useful not only for curtailment of fertility but also the reverse, namely promotion of fertility, which when applied to animal production will be of economic benefit. Better understanding of the mechanisms by which the conceptus carrying 50% of paternal genetic information foreign to the mother, is not rejected, will have implications in transplantation biology. The common links between embryonic and oncogenic proteins have the potential of diagnostic and therapeutic applications in tumour biology.

II. HYBRIDOMAS: AN ABUNDANT SOURCE FOR ANTIBODIES OF CONSISTENT CHARACTERISTICS

Although the principle of fusion of somatic cells to generate hybrids with genetic capabilities of both partners was experimentally evolved two decades back, as a result of the work of Boris Ephrussi and Henry Harris, it was only eight years back that Kohler and Milstein[18] used this principle to create hybrid cells making antibodies of defined specificity. Within a few years, the method has been widely adapted to create clones of hybrid cells making antibodies of selected type against a wide variety of antigens.

Our laboratory was amongst the first to generate hybridomas making antibodies against some reproductive tract hormones and a protein present in a layer surrounding the mammalian egg. The procedure followed[19] was simple, which can easily be adopted by many other laboratories in the country. We shall elaborate a little on the properties of these clones, not only because it is a work the present author is most familiar with, but also to point out in an illustrative manner the diverse uses to which these antibodies can be put.

Monoclonal Antibodies against hCG

Human chorionic gonadotropin (hCG) is a hormone characteristic of pregnancy. It is made by the implanting trophoblast at a rather early stage and has long been employed as a marker for diagnosis of pregnancy. Diagnosis can be made by a number of methods based on bio-

logical and immunological properties of this hormone. The biological methods are cumbersome and are largely displaced by the latter. In all immunological methods, one of the most important ingredients is the antibody, which should have a high specificity for recognition of the putative antigen *e.g.* hCG in this case with non-reactivity with other hormones and constituents of the urine. This is precisely what has been accomplished by a clone which we have developed against this hormone[20]. The product of the clone P_3W_{80} has high affinity for hCG ($Ka = 3.03 \times 10^{10}$ L/M). It recognizes best the native hormone, and the β-subunit of the hCG, has low reactivity with alpha-hCG and has for all practical purposes no cross-reaction with the pituitary hormones; hLH, hFSH and hTSH. Within the hCG and β-hCG, its binding is not with the tail end but with a determinant present in the core part of the molecule. This clone has been adjudged by scientists and industry to be one of the best available anywhere in the world and one can record with satisfaction that after due evaluation, it has been sought and acquired by a leading US firm for incorporation into their range of products radioimmunoassay and other types of diagnostic kits. We are hopeful that it will also be of utility within the country, where it was developed. The delay in adopting this technology within India is perhaps related to the degree of development of the immunodiagnostic industry in the country. Very few firms are ready to launch out products for which a complete package is not ready and saleability not demonstrated. This is perhaps the reason for our dependence on imported knowhow and technology. There is a gap between the stage to which research laboratories develop a lead and its industrial utilization. Some one has to do the product development. Either the research laboratories in this area have to add a complement to carry forth the basic lead through the stages of product development or the industry has to pick up the lead from the laboratory, as is done in industrialized countries, and to take it to its logical conclusion through the intermediary stages. A close liaison between universities, research laboratories and industry in countries such as USA, Japan, Germany has proved the economic utility of the link-up and advantage to both sides. The research laboratories can get funds for their work from non-governmental sources by this channel; they can also have access to a type of organization which industry possesses and which is required for some research undertakings. For example, without the organizational backing and cooperation of Scherring for the supply of the very large quantities of urine and its concentrates, the discovery and elucidation of the chemical structure of the sex hormones by Butenandt may not have been that easy. Functional liaison between industry and research laboratories is a culture which needs to be inculcated in a fuller sense for a country seeking the utilization of science for economic uplift.

Simple kits for Diagnosis of Pregnancy and hCG Synthesizing Tumours

Realizing the present gap, we have gone forward to make prototype of diagnostic kits[21,22] which use simple raw materials mostly available in the country. The methods are more sensitive than the previously available commercial kits based on latex agglutination. They enable the detection of pregnancy within the first week of the expected menstrual period instead of the 13–15 days after the missed period around which the latex agglutination methods become positive. There are no false positives because of the quality of the antibody and the principle employed. The urinary constituents do not interfere in the assay. The proposed methods are clearly readable by virtue of the distinct colour change, which is deep blue to white or white to yellow depending upon the enzyme and the modality used. Two other properties are worth noting: the reagent cost is low and the kits are stable for prolonged periods at room temperature, avoiding thus the requirement of refrigeration facilities, which may not be available in far flung areas and even in cities with irregular supply of electricity. The methods are amenable to qualitative and quantitative use. These can be employed for the diagnosis of pregnancy, and

also for detection of tumours making hCG. It may be recalled that besides trophoblast hyperplasia, choriocarcinomas, hydatidiform moles, hCG is also synthesized and secreted by a variable proportion of nontrophoblast tumours[23] such as testicular tumours (37–66%), adenocarcinoma of ovary (38%), breast cancer (18%), lung cancer (11%), carcinomas of gastrointestinal tract (21%). A serial follow-up of samples from patients carrying the hCG synthesizing tumour can be helpful in (a) confirmation of the total removal of the tumour by surgery, (b) the efficacy of chemotherapeutic regime and (c) appearance of metastasis.

New Dimensions of Immunodiagnostics

Hybridoma technology can make available highly specific antibodies in abundant amounts. The clones once developed are a far cheaper source than the traditionally employed animals (horses, rabbits, etc) for producing antibodies. Stabilized clones can further ensure constancy of the quality of the product avoiding the batch to batch variations inherent in conventional methods. These are traits suitable for industrial undertaking, and one can forsee a rapid growth of immunodiagnostics as an industry. Another important development in this field is the successful use of non-isotopic ligands as tracers instead of the radioisotopes. In many cases these assays have attained the sensitivity of determination in the nanogram and picogram range. The non-isotopic immunoassays do not require expensive equipment or dependence on the supply of radioisotopes. Immunodiagnostics is a sort of "appropriate technology" that can be practiced in the laboratory and in the field. It is likely to be of service to a large number of people in the developing countries. It may also be useful in industrialized countries and replace radioimmunoassays, owing to the advantages of lack of radiation hazard to workers and freedom from disposal of radio-active wastes. It is today possible to employ the non-isotopic methods for qualitative and quantitative determinations of hormones, drugs and a variety of bacterial, viral and parasitic antigens. A collection of papers on the subject has recently been published[24], which gives an idea of the wide range available.

Diagnosis is an important aid to rational management of a case and therapy. The non-isotopic immunoassays, of which enzyme linked assays constitute a large part, are fairly rapid methods and easy to perform. Their high sensitivity can permit early diagnosis. They should displace many of the traditional tests employed in hospital and health laboratories. In some cases they may be of far reaching importance. An example follows:

Leprosy—Desirability of Early Detection

Leprosy is caused by an acid fast bacillus Mycobacterium leprae discovered by Armeur Hansen about 110 years back. This bacteria has not been grown *in vitro* convincingly eventhough papers appear now and then claiming the same. As far as one knows, man is the major reservoir of the infection in this country. Other living species in which it can grow are (i) the nine banded armadillo, a peculiar animal native to Latin America and Southern USA (ii) the Chimpanzee and (iii) an African species, the Mangabey monkey and perhaps the rhesus[25]. A small percentage of human beings (less than 1%) with deficient immunity cannot overcome the infection of *M. leprae* and develop in course of time the lepromatous leprosy. These are the individuals who constitute a propicious soil for growth and proliferation of these bacteria and serve as a foyer for transmission to others. Leprosy has a long latent period and it may take 5 years or more for the clinical symptoms to manifest. During the incubation period, bacillus multiplies in the organs of these patients. Their nasal droppings are loaded with bacteria and it is reported that a sneeze at close contact, can transmit enough bacilli for infection of a mouse[26]. The bacteria from nasal discharge stay viable for several weeks in dust[27]. It is obvious that unless steps are taken to contain the infection, the eradication of the disease may not be possible. The present control programmes focus on detection of clinically manifest cases and their treatment. However, in spite of commendable

Table 3 *Prevalence rate of leprosy in three districts surveyed after 37 years*

District	Prevalence rate per 1000		Lepromatous rate (%)	
	1944	1981	1944	1981
Warangal	3.0	16.2	47.0	12.0
Karimnagar	2.4	10.8	23.5	11.0
Adilabad	3.0	10.5	28.5	13.1

Data from Christian[48]

work by the government and voluntary agencies, the prevalence of the disease has not shown a decline (table 3) and the total number of leprosy patients in the country has registered a continuous increase over the last 40 years. Furthermore, leprosy has spread. It is no longer endemic in the southern, or eastern parts of the country, but cases are found in Rajasthan and other states which were formerly free of the disease. The rootcause is the transmission of the bacillus from those carrying it to others in the community. How soon the carriers become infective is not known. For an effective control programme, a method for putting in evidence a carrier in a subclinical state would be extremely valuable. This is perhaps possible, as experimentally infected armadillos, leprosy patients and family contacts exposed to infection are demonstrated to carry antibodies in their blood to *M. leprae*[28] The utility of the fluorescent antibody test to identify the patients and their contacts has been reported.[29,30] This test is not positive in healthy people living in non-endemic areas. This is a good indication of the feasibility of the approach. The fluorescent antibody test is, however, cumbersome and needs to be replaced by an enzyme immunoassay which can be used more widely. Several laboratories including ours are engaged in developing visual colour assays for detecting anti-*M. leprae* antibody. Again here, hybridomas making antibodies specific to *M. leprae* are most useful and indeed success has been registered in making such clones[31]. Leprosy is a high priority national health programme and it can be hoped that by the encouragement and support being given to research in this field within the country, simple methods will be established for putting in evidence the antibodies and antigenic components in leprosy. In a disease such as this, a diagnostic method, providing evidence for subclinical infection, is of extraordinary importance, as with the help of available bactericidal drugs, infection can be cleared and its transmission prevented to others. This would therefore be a valuable component of the containment and eventual eradication programme.

Hybridoma Antibodies for Therapeutic Purposes

(a) *Sero-therapy for acute life saving situations*: As antibodies are the effective components of immunity in several situations, their production can either be elicited by vaccines within the system or they may be administered for external sources as a therapeutic regime. Antisera raised in horses have been used since several decades for the management of acute cases of diptheria, tetanus and snake bite. The possibility of obtaining almost pure, high titre antibodies by hybridoma suggests an alternate and perhaps a cheaper source for such antibodies. A single monoclonal may not, however, suffice, though it is possible to obtain clones with neutralization ability. Combination of more than one clone is likely to be more potent for the task.

(b) *Control of fertility by passive use of antibodies*: The feasibility of regulating fertility by use of antibodies against hormones and reproductive tract antigens has been amply demonstrated by a number of investigators[17]. The advantage of using preformed antibodies is to ensure the delivery of an amount adequate for effective intervention. The quality of antibodies with respect to bio-neutralization potential can also be controlled. We shall describe briefly the possible applications for fertility control of some of the monoclonal antibodies developed in our laboratory in recent years.

(i) Anti-LHRH: LHRH is a decapeptide normally made by the neurons but observed now to be also made by other tissues such as the placenta[32,33]. A.V. Schally and R. Guillemin were awarded the Nobel Prize for elucidating the primary structure of this hormone. LHRH is a key molecule for

control of both male and female fertility. Hybridoma antibodies in our possession are of reasonably high affinity[34]; Ka = 1.2×10^9 L/M. Leaving aside the wide range of reproductive functions effected by these antibodies in laboratory animals, one of their potential application is in control of estrus in domestic pets. A single injection of the antibody suppresses the estrus in a female dog[34].

Anti-LHRH antibodies were also effective in terminating pregnancy in baboons[35]. The effect is primarily exercised at an early stage of pregnancy. Animals thus terminated return to normal cyclicity after the procedure and can conceive and engender a normal progeny. The action of these antibodies is very similar in substance to that brought about by administration of anti-hCG antibodies generated by the vaccine Pr-β-hCG-TT[11]. The mechanisms by which anti-LHRH interfere in pregnancy is not fully known but the fact that chorionic gonadotropin levels fall after administration of these antibodies, is indicative of the possible relationship between LHRH and hCG. Some investigators have reported the stimulation of hCG production by LHRH[36]. The effect of anti-LHRH antibodies requires to be studied in other species of animals. In mice they prevent progression of pregnancy when given on day 4 or 7 but are without effect if given after day 12. Toxicology studies are in progress and in case they do not indicate side effects, they could be studied in probing clinical trials. They can provide a non-surgical, medical method for termination of pregnancy. Till lately we did not proceed in this direction on the belief that mouse hybridomas may not be suitable for repeated administration in humans for fear of sensitization againt the mouse globulins. Recent observations discussed in the Armand Hammer Symposium in January 1983, however, indicate that mouse hybridoma antibodies given intravenously to humans in amounts of 366–625 mg do not produce anti-mouse antibodies and are thus appropriate for human therapeutic purposes[37].

(ii) *Anti-Zona pellucida*: The mammalian egg has a transluscent layer around it, which is made up of glycoproteins. Antibodies against the zona glycoproteins bring about a physical change in the appearance of the zona. The antibodies prevent the attachment of sperm and impose a block on the fertilization of the egg by sperm. They also prevent lysis of the zona which is needed for implantation of the blastocyst in the uterus.

A single injection of the anti-zona antibodies in the mouse blocks fertility of the animal for about 6–8 cycles. Work in progress in the laboratory suggests a similar long term block of fertility in monkeys by the anti-zona antibodies. The interesting feature in these studies is the long term effect obtained with a single administration of the antibody. A passive approach attains here the advantages of an active approach, in terms of the duration, while retaining the superiority over the latter in ensuring efficacy by virtue of delivery of the required amount of the antibody. We have six stabilized clones making anti-zona antibodies. These are potentially valuable for regulation of fertility. Studies must continue to determine whether the method is otherwise safe and free from side-effects.

(c) *Hybridoma antibody for imaging of tumours and delivery of drugs*: Hybridomas can be generated against proteins and other antigenic moieties present on the surface of cancer cells. We have reported elsewhere the utility and limitation of the antibodies against hCG for localization and immunotherapy of 'BeWo' chorio-carcinoma cells[19]. The antibodies are bound to only a subpopulation of cells carrying hCG on the cell surface, as visualized by immunoenzymatic probes. This may suffice for imaging purposes as indeed Dr Bagshawe, has been able to use anti-hCG antibodies for localization of hCG synthesizing tumours in clinical cases[38]. Ideally, however, the marker to which antibody homes should be such that it is present on nearly all cancerous cells, and absent in the normal somatic cell. Search for such markers goes on. Transferrin receptors are reported to be present on nearly all types of cancer cells.[39] These could constitute a common marker for the tumour cells. The limitation is the fact that they are also present on normal dividing cells. Their number and density is, however, very low in

resting differentiated somatic cells.

Another antigen of interest which is present on most malignant tumours and absent from the somatic differentiated cells (except epithelial cells of urinary bladder, placental villi, skin of 10 week old embryo and kidney) has been described. It is heat stable, resistant to organic solvents, resistant to trypsin and hyaluronidase and sensitive to neuraminidase[40]. This marker can serve as one of the potential targets and indeed monoclonals against it have been developed. Present studies have not identified the ideal and the unique marker of cancer cells but some candidate markers near to that have been discerned. Antibodies against these can be tagged with ^{125}I, ^{131}I and ^{99m}Tc to enable scanning of tissue mass containing tumour cells.

Drug delivery

Another important application of hybridoma antibodies directed against surface markers is their use for delivery of drugs to the malignant cells. The antibody serves in this case as the homing device. Indeed a far better survival rate is observed in experimental mice given Daunomycin loaded on monoclonals raised against mouse lymphomas as compared to those receiving the drug alone[41]. The era of the magic bullet in chemotherapy has perhaps begun, with the possibility of (a) reducing the cost of the amount of the drug required, (b) diminishing substantially the systemic toxicity consequent upon the generalized distribution of the drug in the body as per conventional therapy and (c) better efficacy of the procedure.

Dr Ronald Levy and his collaborators have used mouse monoclonals against Leu-1 antigen to treat human patients suffering from leukemia[42]. A single intravenous administration of the antibody led to a significant improvement in the clinical conditions of the patients.

Anti-Idiotypes

Monoclonals are homogeneous antibodies and carry therefore a given type of idiotope. Their repeated administration can give rise to the formation of antibodies against the idiotope, the anti-idiotopes. These would be the mirror images of the conformation of the idiotope, with complementarity for binding with the idiotope. If the antigen against which monoclonals were raised was a hormone, the antibody to it has the steric shape of its receptor. An antibody to that creates the stereo confirmation of the hormone with ability to bind and activate the target tissue receptor in a manner similar to what the hormone does. It is obvious that such molecules would be biologically active. Monoclonals against monoclonals can thus serve theoretically as an alternate source of biologically active hormones, antigens and drugs.

The idiotypic network is also of regulatory significance and such antibodies can act as specific suppressant or stimulator of a specific arc of the immune response. Who knows, the most elegant adjuvant for an antigen may be an anti-idiotype.

CONCLUDING COMMENTS

We are at the threshold of a new era in immuno-biotechnology with promise of important applications for human welfare. The first products of hybridoma technology have gone beyond the research laboratory and are in industrial use. The innate advantages of monoclonal antibodies, namely the possibility of production in abundant amounts of almost pure, high titre antibody with constancy of characteristics, render them suitable for large scale use. Their applications in diagnosis and therapy are likely to increase significantly. Sensitive immunodiagnostic methods employing non-isotopic ligands have been developed for hormones, drugs and many bacterial, viral and parasitic antigens. They are serviceable in the laboratory and in the field. Their relative low cost and thermal stability favour their widespread adoption. In diseases like leprosy, a method putting in evidence the subclinical infection can help greatly in containment of the foyer of infection.

Therapeutic use of mouse myeloma derived hybridoma antibodies has so far been restrained owing to the fear that their repeated use may sensitize against heterospecies proteins. Recent

observations, however, indicate the lack of such sensitization by administration of antibodies in amounts larger than 350 mg by intravenous route. If these reports are confirmed by experience of others, an important avenue will be opened up for therapeutic use of these antibodies in humans. Amongst the many potential applications would be (a) their use as a nonsurgical or medical method for menstrual regulation and termination of early pregnancy, (b) suppression of estrus in domestic pets, (c) block of fertilization of the mammalian egg for several months (d) their use as a vehicle for imaging of metastasis and delivery of drugs to cancer cells.

Vaccinology is entering a new phase. Hazards cannot be completely eliminated in vaccines based on attenuated organisms, in certain situations they can become virulent. Mutants can also emerge. Accidents have also taken place in the past, though rarely, in production batches of killed vaccines. These can all be excluded by use of an isolated protein with capability of conferring immunity instead of the total organism. Such proteins have been identified for several pathogens in recent years and cloned by DNA recombinant technology.

This second generation of vaccines is being rapidly succeded by another trend, the search for sub-molecular domains of the protein molecule carrying the essential information. Indeed peptide sequences of relatively short length (10 to 30 aminoacids) have been identified in bacterial and viral key proteins. Elegant computer graphic techniques have been refined to project determinants present on the surface of the protein molecule which are recognized by the immune cells. These are then synthesized chemically. In some cases, they have been observed to be more immunogenic than the whole molecule. Their stability is better and they would be safer to use. Synthetic vaccines may thus well replace the traditional vaccines for some diseases within this decade.

Recent research has also pointed to the feasibility of another set of novel vaccines—the Structured Vaccines, devised for control of fertility by linking a hormonal sub-unit with a bacterial carrier such as tetanus toxoid. These vaccines are designed to make antibodies simultaneously against the two partners with potential double benefit. This principle could be utilized to make polyvalent vaccines with multiple benefit in the coming years.

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