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(*e.g.*, H-atom) in glycine [H_2N-CH_2-COOH] is an absolute necessity to enable and execute the phenomenon of the folding pattern.

4.5.1.4.3 Glycosylation of Antibody (IgG)

Generally, an '*antibody*' gets **glycosylated**, invariably carrying carbohydrate residues specifically located in the CH_2 region. It has been established that the carbohydrate portion of the Ig is essentially an oligosaccharide loaded with several '*monosaccharide units*'. It may include rather **complex N-acetyl lacto amine rich segments** as depicted in Fig. 1.23 given below:

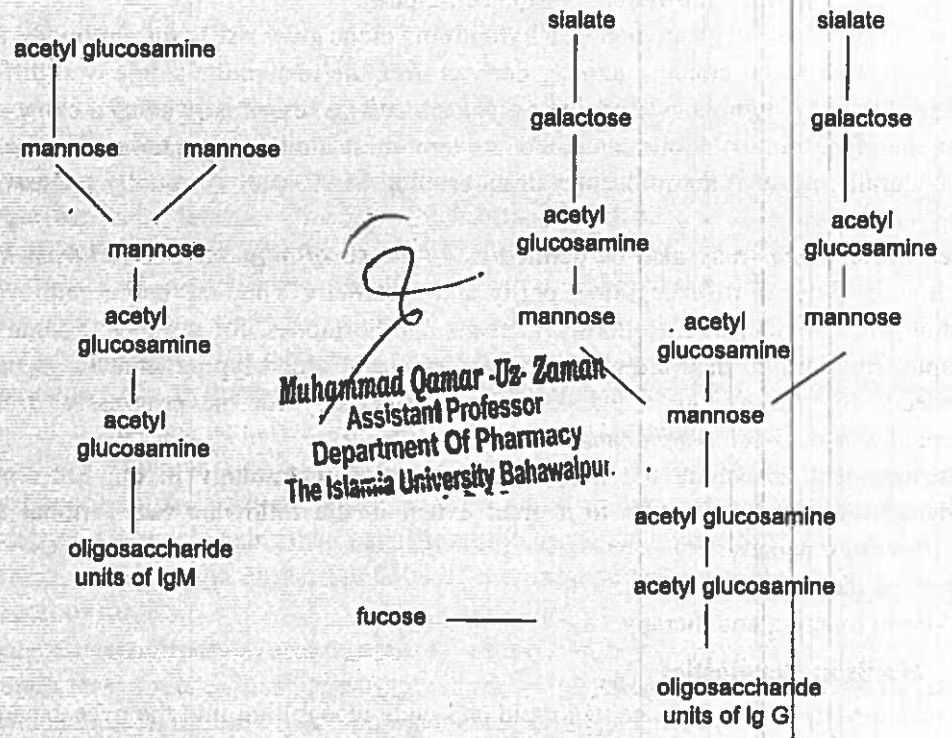

Fig. 4.23 *Structure of Carbohydrate Moieties (Oligosaccharide Segments) Observed in the Antibody IgG.*

However, it has been found that in IgG, the presence of the '*carbohydrate segment*' extends upto 2.5% of the entire molecule ; and, besides, it is practically equally divided between the prevailing **two γ- heavy chains (H),** that are strategically linked *via* two specific *amino acids*, namely: **threonine** and **aspartic acid** residues present in the **polypeptide chains.** It has been adequately proved that there exists no *'glycosylation'* in the fragment antibody binding (F_{ab}); and this perhaps puts across a logical explanation that glycosylation exerts practically little effect on the **antigen binding property of antibody.**

However, it affords an influence upon the effector function duly controlled particularly by the fragment crystallisable (F_c) component.

4.5.1.5 Monoclonal Antibodies (MABs)

In a broader perspective, an antigen (or immunogen) molecule predominantly possesses antigenic determinants of more than one specificity. In other words, different determinants shall undergo viable interaction with altogether different antibodies. In reality, each separate antigenic determinant of the antigen will have a tendency to get bound to a fully mature B-cell whose surface immunoglobin (SIg) specifically matches the characteristic features presented by the concerned determinant. Consequently, a single antigen thus produced may essentially activate the B-cells having more than one SIg specificity. The resulting activated B-lymphocytes (cells) of each SIg specificity *shall*

precisely *divide* and *differentiate* to produce **clones** of the respective plasma cells thereby generating antibodies having more or less the same specificity. Interestingly, it has been observed that a '*single antigen*' would usually induct more than once distinct clones of the prevailing plasma cells; and, therefore, it will give rise to the production of **'antibodies'** bearing variant specificities. Most logically, the serum of an animal adequately immunized by a single antigen shall definitely comprise *antibodies* with *various specificities,* but **reacting particularly to the same antigen.** These specific varieties of antibodies are invariably termed as **polyclonal antibodies** because they are eventually produced by a good number of different plasma cell-clones.

Contrary to this aforesaid phenomenon, a **hybridoma** clone gives rise to the antibodies of a single specificity as the particular clone is actually derived from the fusion of a single well differentiated (antibody producing) B-lymphocyte having a *mycloma cell i.e.,* essentially being a clone of a single Bcell. It is; therefore, quite obvious and evident to term these antibodies as **monoclonal antibodies** (MABs). Naturally, most of the molecules of an ensuing MAB shall essentially possess the **same specificity.**

In other words, MAB may also be defined as – '*a type of antibody derived from hybridoma cells*. Such antibodies are of exceptional purity and specificity. They are being employed for the identification of a plethora of infectious organisms and hormones, for instance: human chorionic gonadotropin. In addition, they are employed in tissue and blood typing (matching), in order to identify specifically the tumour antigens, and experimentally for the progressive treatment of *autoimmune diseases, B-cell lymphomas, and pancreatic cancer.*

The astronomical growth in the field of **pharmacobiotechnology** in the last two decades has broadened the scope of MABs to a great extent in the following *two* cardinal aspects of *immunodiagnostics,* namely:

(*a*) MABs in diagnostics, and

(*b*) MABs in imaging and therapy.

4.5.1.5.1 MABs in Diagnostics

In the recent past, MABs have gained rapid and wide recognition into the ever expanding field of heath-care diagnostics. In fact, there are normally *four* vital and predominant methodologies that find their enormous applications in '*diagnostics*', for example:

4.5.1.5.1.1 Immunoassays

Most immunoassays are carried out by the application of radioactive antibodies [*i.e.,* radio immunoassays (RIAs)] whereby the sample exhibiting radioactivity shall be retained onto the sample.

However, the underlined and prescribed stringency and authencity of RIA tests largely restrict it to centralized specialist diagnostic facilities exclusively.

4.5.1.5.1.2 Enzyme Immunoassays (EAI)

In this specific instance a particular colour-producing enzyme is coupled to the antibody. Thus, the outcome of the results may be read either directly by a naked eye or spectrophotometrically.

4.5.1.5.1.3 Enzyme Cascade Technique

Here, a number of enzyme reactions are taking place are coupled strategically to produce an appreciable amplification of the original binding signal that is either read by a naked eye or spectrophotometrically, and

4.5.1.5.1.4 Fluorescence Immunoassays (FIA) and Luminescence Immunoassays (LIA)

Precisely, these are more or less inter-related techniques wherein the **'lable'** either gives rise to fluorescence or light respectively.

Examples:

1. **Pregnancy Dipstick Test.** It is solely based on MABs ; the pregnancy dipstick test determines the pregnancy either at home or in a clinical laboratory.
2. **Ovulation Dipstick Test.** Another type of dipstick test based on MABs that essentially ascertains the positive or negative ovulation on a subject, and
3. **AIDS test.** MABs based AIDS test kit is abundantly available to identify its presence in donated blood samples.

Notes. *Therefore, each and every blood sample must be tested for AIDS test before the actual blood-transfusion is carried out onto a healthy patient.*

4.5.1.5.2 MABs in Imaging and Therapy

It is, however, quite pertinent to state here that the most acute and major observed hinderances ever encountered in the management and subsequent treatment of cancer virtually lies in the fact that the *malignant cells* have a very close resemblance to the *normal cells*. Therefore, it is quite evident and possible that such '*therapeutic agents*' which are solely intended to cause complete destruction of the cancerous cells would also destroy invariably the '*normal cells*' as well perhaps by virtue of their close resemblance. However, it has already been well established that the surfaces of the malignant cells do differ in certain respects from those of the normal cells. But we have seen earlier that MABs exclusively recognize specific *antigens* on cells, they ar being fully *exploited to image* cancerous tumours particularly in an intense on-going clinical research undertaking, and also in therapy against a variety of malignancies, namely: colon and breast cancer; lymphomas; and melanomas.

A few typical examples have been adequately detailed below, namely;

1. **Gastrointestinal Cancer.** MABs is used alone to combat gastrointestinal cancer. The underlying principle being that when the antibodies opt to bind to the turnover, they invariably exhibit a tendency to attract the cells of the immune system to act against the prevailing cancerous tissue.
2. **Lung, Breast, Prostate, and Pancreas Cancer.** It is, however, pertinent to mention here that enough research activities have triggered off in the recent past towards the development of monoclonal conjugates of *two* important class of drugs, such as:

i. Anthracycline Drugs. Such as antibodies having quinones and related structures *e.g.*, Adriamycin(R) (Adrio); Bufex(R) (Bristol).

ii. Desacetyl Vinblastine. When desacetylvinblastine *i.e.*, a chemical entity obtained either from the plant source or produced by plant cell culture, is conjugated to a monoclonal which consequently acts specifically on lung, prostrate, breast and pancreas malignant cells.

4.5.1.5.3 Production of Monoclonal Antibodies (MABs):

It is well established at present that – *'monoclonal antibodies are invariably produced from hybridoma clones ; whereas each hybridoma clone is meticulously derived by the actual fusion of a mycloma cell together with an antibody producing lymphocyte, and ultimately the hybridoma clone producing the desired antibody is adequately isolated and subsequently identified.'*

In actual practice the *'hybridoma cells'* are **mass cultured** for the overall production of MABs with the help of one of the following two methods, namely:

(*a*) Culture in Peritoneal Cavity *i.e., in vivo* peritoneal cavity of mice, and

(*b*) Mass *in vitro* culture *i.e., in vitro* large scale culture vessels.

The above *two* methodologies shall now be discussed individually in the section that follows:

4.5.1.5.3.1 Culture in Peritoneal Cavity

In this developed, tested and tried methodology the '*hybridoma cells*' are strategically transplanted into the peritoneal cavity of a suitable and highly purified strain of mice, and subsequently the *ascitic fluid* derived from the animals is duly harvested and the MABs are purified meticulously.

Importantly, this particular technique positively yields between 50-100 times higher quantum of the **'desired antibody'** in comparison to the usual traditional in vitro culture of the hybridomas.

It is however pertinent to state here that there are *three* important characteristic features of this technique, namely:

(*a*) Generally, the ensuing '*antibody preparations*' happen to be a **lower purity** than those obtained from the corresponding cell cultures, particularly if, **serum-free media are employed,**

(*b*) Methodology involved is predominantly a **labour-intensive** one, and

(*c*) Unconditionally and absolutely **pathogen-free animals of particular genotypes** are essentially required.

4.5.1.5.3.2 Mass in vitro Culture

One may accomplish the commercial/large-scale culture of the *'hybridoma cells'* by adopting any one of the *three* methodologies, namely: (*a*) *Bioreactors with frequent stirring device ; (b) Aircraft fermentors;* and (*c*) *Specific vessels based on immobilized cells.* In actual practice, the culture systems making use of specifically **immobilized cells** are responsible for the progressive cultivation of cells at very high densities that markedly increases the production of **'antibody'** *in vivo.*

Examples:

There are *two* typical examples to expatiate the above process i.e., mass in vitro culture, namely:

(*a*) **Hollow fibre cartridges** (*i.e.,* a culture system) – found to yield 40 g MABs *per month*; and

(*b*) **Special ceramic cartridges** (i.e., an optical system) – found to yield 50 g MABs *per day.*

Future Scope. An extensive and intensive research towards the futuristic developments and progress in the area of immobilized culture systems may ultimately give rise to an increased production of MABs in a significant manner and therapy markedly and pronouncedly minimize the cost of their mass production from the cell cultures.

Production. The various steps that are intimately involved in the *production of monoclonal antibodies* (MABs) are represented sequentially in Fig. 4.24 as below:

The various steps that are involved sequentially in the production of MABs and polyclonal antiserum (Fig. 4.24) are as follows:

1. A very specific '*antigen*' (immunogen) comprising of **four epitopes** was injected into mice where B cells have already commenced generating **antibodies** against that antigen.
2. The same mice (pure strain of albino mice), received another **'booster dose'** of the same antigen so as to accomplish a much desired **'secondary response'.**
3. The **'spleen'** of the treated mice was duly removed after a gap of 3-4 days that essentially comprised of B cells active enough in the process of synthesizing **'specific antibodies'.**
4. The isolated spleen was adequately macerated and the resulting *spleen cells* thus obtained in the form of a suspension consisting of B cells giving rise to **four distinct cell lines** *i.e.,* one cell line representing a specific *antigenic determinant (epitope).*
5. The resulting spleen cells were meticulously mixed with the *mycloma cells* of the mice derived from the bone marrow and incubated in a culture medium containing polyethylene glycol (PEG).
6. Quite a few of the *'spleen cells'* were adequately **fused with neoplasm** (*tumour*) **cells** to result into the formation of **hybrid mycloma cells.**
7. The spleen cells thus obtained are **hypoxanthine phosphoribosyl transferase** (HPRT) – positive and fuse with myeloma cells to give rise to hybridomas [see (6) above] ; besides, utilize *hypoxanthine* categorically to generate *purines* and *pyrimidines.*
8. The **hybrid myeloma cells** (*hybridomas*) do survive and continue to multiply indefinitely thereby producing a good number of **'specific antibodies'** against the **'specific antigens'.**
9. Each hybridoma cell is isolated meticulously and duly cultured individually to allow them to multiply in a clone of *daughter cells.*

10. It has been observed that such **'hybridomas'** are absolutely uniform and permanent characteristically; and, therefore, when *cloned through several generations*, invariably give rise to **only one type of antibody** having specific feature of the parent B cell, hence termed as **monoclonal antibodies** (MABs).

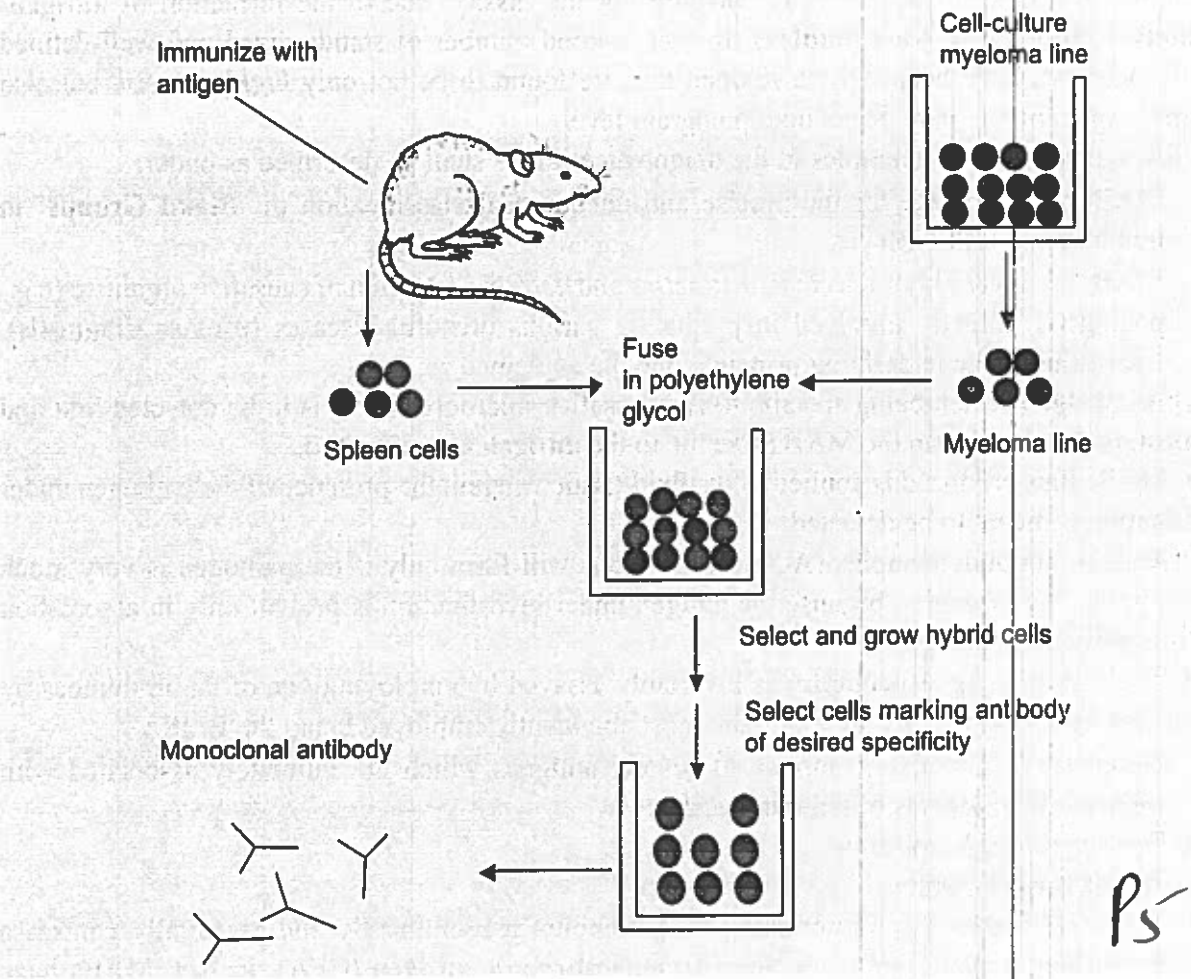

Fig. 4.24 *Production of MABs and Polyclonal Antiserum.*

4.5.1.5.4 Application of Monoclonal Antibodies (MABs)

The most spectacular major advantage of the monoclonal antibodies (MABs) is that most of the antibody molecules present in a *single preparation* strategically undergo reaction with a **single antigenic determinant** or a **single epitope.**

Consequently, the outcome of results achieved by the aid of MABs are not only significant but also explicite and devoid of any ambiguity because there prevails absolutely little confusion which may eventually come into existence by virtue of the presence of antibodies essentially displaying other specificities in the instance of *conventionally employed antisera.* However, in the light of above diversified multiple and wide spectrum applications of MABs, these may be classified judiciously into the following *four* categories, namely:

(*a*) Diagnostic Utilities,
(*b*) Biological Reagents in Diversified Disciplines,
(*c*) Therapeutic Usages,
(*d*) Immunopurification, and
(*e*) Miscellaneous Applications.

These categories of applications of MABs shall now be dealt with separately in the section that follows:

4.5.1.5.4.1 Diagnostic Utilities

Diagnostic utilities are mainly focused when MABs are employed to detect and identify the very presence of either a particular antigen (immunogen) or of antibodies specific to an antigen in a sample or samples. It is, however, pertinent to mention here that the presence of antigen is invariably accomplished (detected) by precisely carrying out the **'assay'** due to the formation of **antigen-antibody complex (Ag-Ab Complex).** In fact, a good number of standardized and well-defined assay procedures have been duly developed that are found to be not only *highly precise* but also *extremely efficient i.e.,* may detect upto picogram level

A few typical specific examples of the diagnostic utilities shall be described as under:

1. MABs are available for the precise and unequivoeal classification of **'Blood Groups'** in humans *e.g.,* ABO, Rh etc.
2. MABs are invariably for a *clear, distinctive* and *decisive* detection of causative organism (*e.g.,* pathogens) directly involved in producing various dreadful diseases (**disease diagnosis**). Interestingly, the underlying principle may be explained as:

(*a*) An **antigen** which being specific to the causative microorganisms is to be detected first and foremost, isolated and than the MABs specific to the **antigen** are generated.

(*b*) MABs thus produced is applied to the fluid/tissue wherein the presence of the pathogen under investigation is meant to be detected.

(*c*) Antigen-antibody complex (Ag-Ab Complex) will form only if the pathogen is very much present in the *'test sample'*, because the antigen under investigation is present only in association with this particular pathogen.

(*d*) The resulting Ag-Ab complex is invariably assayed by employing one of the immunoassay procedures *e.g.,* RIA, ELISA; of which the most abundantly employed being the ELISA

3. Neoplasm's (tumours) comprise of several antigens which are intimately associated with *three* cardinal aspects of tumour, such as:

(*a*) *Tumour cell differentiation;*

(b) Tumour growth; and

(c) Tumour immunology. Importantly, most tumours predominantly and essentially contain a **'marker antigen'** usually known as the **carcinoembryonic antigen** (CEA). In fact, MABs have been specifically produced for CEA together with certain other tumour-linked antigens.

In actual practice, the application of such tailor-made MABs in *histochemical assays* allows the clear-cut identification of such vital information, for instance:

(*a*) The nature of tumour cell type,

(*b*) The malignant and benign king of neoplasm's, and

(*c*) The early instances of *melastasis.*

Note. (1) Radioimmunoassay may detect even small tumours to the extent of 0.5 cm in size that are otherwise not detectable conveniently.

(2) Immunological assays are capable of detection of cancerous cells at a very early stage which is of immense help and advantage in 'cancer chemotherapy'.

4. MABs may be effectively and accurately used for the detection of *'particular chromosomes'* of a given species.

It may be accomplished by adopting the following steps:

(a) Raising MABs against particular proteins that are duly encoded by the genes present in different chromosomes of a specific *viz.,* for the *amylase inhibitors* encoded by the **genes in chromosomes 1 and 6 of wheat.** The actual quantum of Ag-Ab formation in the tissues, such as: seed, extracts, from various individuals may be employed to ascertain in case an individual is found to be:

Nullismic : *i.e.,* **no** Ag-Ab complex formed:

Monosomic : *i.e.,* **low** quantum of Ag-Ab complex formed;

Normal Disomic : *i.e., intermediate* amount of Ag-Ab complex formed;
Trisomic : *i.e., high* quantum of Ag-Ab complex formed.
Specific for the **'concerned chromosome'.**

4.5.1.5.4.2 Biological Reagents in Diversified Disciplines

The most pivotal, major and extremely important major applications of MABs in the capacity of **'biological reagents'** in a number of diversified disciplines are provided in Table 6 as under.

Table 4.6 *Applications of MABs as Important Biochemical Reagents in Diversified Disciplines*

S.No	Discipline	Applications
1.	Bacteriology	Identification of microorganism, and their respective pathogenicity (ie., disease producing organisms)
2.	Cytology	Cell separation by employing flourescent antibodies, carcinoma cells etc.,
3.	Diagnostics	Diagnosis of viral hepatitis, typhoid, filariasis, amoebiasis, breast cancer, HIV-infections, pregnancy tests, haemolytic diseases, genetic disorders, Japanese encephalitis, autoimmune and immunodeficiency disease.
4.	Forensic science (Criminology)	Characterization of Blood stains.
5.	Immunology	Immunoassays, characterization antibody, molecules, antigenic determinants, neoplasm antigens, analysis and identification of T-cell subsets, cytotoxic drug conjugated with MABs against tumour antigens to act as **'magic bullets'.**
6.	Medicine	Identification of blood group, tissue typing, *human leucocyte antigen typing* (HLA-typing), blood clotting factors.
7.	Pathology	Test for allergens in vivo.
8.	Pharmacology	Estimation of drug substances e.g., barbiturates, antibiotics, antineoplastic agents etc.
9.	Virology	Detection and identification of viruses, expression of viral antigens in infected cell-membranes etc.

4.5.1.5.4.3 Therapeutic Usages

The therapeutic usages essentially and prominently make use of MABs to combat *two* vital aspects: *first*, the management and treatment of a disease condition; and *secondly,* to afford a reasonable protection from a disease profile. A few typical examples eliciting certain exemplary developments in this specialized field are enumerated as under:

a. Immunotoxins with 'Ricin'. Antibodies specific to neoplasm cells (i.e., a cell-type) may be linked with a particular toxin polypeptide thereby giving rise to a conjugate molecule normally termed as **immunotoxin.** It has been amply demonstrated that the antibody segment of the prevailing immunotoxin shall be strategically bound to the **'target cells';** and, therefore, the attached toxin will categorically kill the ensuing cells. Interestingly, the immunotoxins with **'ricinin'** have been prepared successfully and evaluated subsequently by accessing their ability to kill the *'neoplasm cells'* with commendable success. The resulting toxin is observed to be very much effective against both *dividing* and *non-dividing* cells because it helps in the inhibition of protein synthesis to a considerable extent. Importantly, the conjugate derived from **antibody-Ricin A** has been shown to reduce protein synthesis particularly in mouse B-cell neoplasm's. Besides, the antibody employed in forming the conjugate was found to be absolutely specific to the '*antigen molecules*' present on the surface of the prevailing target neoplasm cells as shown in Fig. 4.22.

Note. The '*immunotoxin with Ricin*' failed to exhibit any binding affinity to either other neoplasm cells or the normal cells.

b. Radioactivity to Target Tumour Cell. Based on the identical principle it has been adequately exploited to deliver radioactivity particularly to the target tumour cells. Interestingly, in this specific

instance, radioactivity caused by virtue of ^{131}I (iodine), ^{90}Y (yitrium), ^{67}Cu (copper), ^{212}Pb etc., is strategically incorporated (or inducted) right into the neoplasm specific antibody (*i.e.,* **toxin is not used**). Consequently, the prevailing **radioactive antibody entity** exclusively gets bound to the tumour cells that in turn *express the particular antigen.* Thus, the radiation ultimately emitted by the isotopes helps to kill the neoplasm cells and also their neighboring cells.

Broadly speaking, this particular approach is commonly known as **'radio imaging'** to solely detect *neoplasm cells* for which the *antibodies* are more or less extremely specific.

Examples:

A few typical examples of the radio-labeled antibodies that have been used extensively as therapeutic purposes are:

For Hepatoma: Human T-cell leukemia/lymphoma virus-1 (HTLV-1); and Adult T-cell leukemia (ATL).

c. The proper activation of T-cells (lymphocytes) by virtue of their adequate *proliferation, maturation* and *antibody secretion* achieved due to their **interleukin-4 [IL-4] dependence profile.** Perhaps the aforesaid observation would certainly go a long way to put forward a solid explanation that both *tissue* and *bone marrow* explant rejections are significantly mediated by T-cells. Therefore, quite evidently an overwhelming strategy to lower the probability of rejection of '*grafts*' from other individuals (*i.e.,* **allograft**) in particular shall be aimed at to eradicate the T-cells from either bone marrow or circulatory system (*viz.,* blood stream) by employing **T-cell specific MABs.** In general, T-cells do display many *antigens* (immunogens), but it has been observed that CD3, CD4, CD8 etc., have been the most preferred targets for the development of MABs.

Methodology. The various steps involved in the bone-marrow transplantation are enumerated briefly as under:

1. Bone marrow cells of the recipient are adequately inactivated by appropriate radiation.
2. Donor bone marrow cells are meticulously subjected to the T-cell specific antibodies to cause destruction of the T-cells present in them; and, subsequently, the residual treated cells are transplanted into the recipient.

d. Passive Immunity against Diseases. MABs may be employed to cause an efficacious and preventive '*passive immunity against diseases*'. It has been squarely proved that the **'active immunity'** duly inducted in an immunized individual by itself generates the antibodies against the concerned pathogenic microorganisms (*pathogens*); whereas, interestingly in the specific instance of **'passive immunity'** antibodies that are actually produced elsewhere are adequately introduced into the body of an individual to make the **required and desired provision of immunity** against the *concerned pathogens.*

e. MABS are found to be extremely beneficial in affording the purification of antigens that are particularly specific to the '*concerned pathogens*'. In short, these '*highly purified antigens*' are invariably employed as **vaccines** *e.g.,* polio vaccine, cholera vaccine, small pox vaccine etc.,

4.5.1.5.4.4 Immunopurification

The highly specific and critical interaction of an *'antibody'* to an '*antigen*' is largely employed for the purification of antigens that are essentially present in small quantum in the form of a mixture along with several kinds of other molecules; and this phenomenon is termed as **'immunopurification'**.

The various kinds of immunopurification usually encountered are discussed as under briefly:

1. The specific structure of a *MAB molecule* should be largely compatible to the antigen that needs to be purified; and the latter is invariably fixed to an *insoluble matrix,* such as: *dextran* [Macrodex$^{(R)}$] or *agarose beads, strategically joined together by a cross-linking* agent like *cyanogens bromide* in such a fashion that its **inherent antigen-binding** ability is least affected. The aforesaid beads are suitably packed into a column *via* which the solution consisting of the '*antigen*' is made to elute under standard specified conditions. The *antibody molecules* present in the system do interact

appropriately with the *antigen molecules* thereby giving rise to the formation of Ag-Ab complex that is obviously held up in the column whereas the residual molecules (smaller in size) get eluated rather freely without any inconvenience whatsoever. Now, appropriate washing processes are adopted so as to collect the **'purified antigen'** retained as Ag-Ab complex in the column. This prevailing technique is commonly known as **'affinity chromatography'.**

Note. *Exactly the reverse of the above phenomenon is skillfully used for the purification of MABs i.e., in this specific instance the 'purified antigen' is duly fixed onto the beads adequately packed in a column via which the antibodies are passed. Consequently, the MABs from the ensuing Ag-Ab complex are meticulously recovered in its purest form.*

2. MABs have been frequently utilized in the isolation of mRNA* via encoding the particular protein entity to which the MABs are eventually specific.

Methodology:

The different steps involved are as follows:

(*a*) Almost one dozen of *ribosomes* are intimately associated with the *'active protein synthesis' in vivo* being supported by one mRNA molecule. Consequently, one ribosome is closely linked to a molecule of the corresponding *polypeptide* undergoing synthesis; and this very resulting new structure is usually termed as polysome.

(*b*) The ensuing '*preparation of polysomes*' on being treated with antibodies, undergo instant interaction with the *highly specific nascent polypeptides* closely linked with the ribosome's thereby affording ultimate precipitation of the prevailing **'polysomes'.** This phenomenon evidently suggests that a specific MAB shall only precipitate such '*polysomes*' that are critically engaged in the synthesis of **polypeptide** for which the MAB is articulately specific.

(*c*)Subsequently, the **'precipitated polysomes'** are normally recovered by standard procedures, and the mRNA is isolated carefully.

(*d*) The resulting mRNA is found to be extremely pure in nature; and, therefore, predominantly aids in the process of encoding the protein for which the specific MAB was initially engaged in the precipitation of the '*polysome*'.

3. MABs are invariably utilized for several vital operations, such as: identification, isolation of cells exhibiting a particular 'antigen' on their surface.

Methodology: The various steps involved are as stated below:

a. MAB which is specific to the *'concerned antigen'* being articulately conjugated with a fluorescent molecule.

b. The MAB very much specific to the concerned antigen is conjugated with a *'fluorescent molecule'*; and thereafter, added to the corresponding *cell suspension* to facilitate Ag- Ab complex production.

c. The resulting '*antibody conjugate*' shall ultimately get bound to those cells only which prominently display the 'concerned antigen on their surface; and, therefore, such cells will exhibit fluorescence under suitable conditions.

d. In actual practice, these specific cells are easily identified and subsequently separated from the others by virtue of their '*fluorescence characteristics*'. Hence, for this precise measurement one may make use of highly sophisticated **'fluorescence activated cell sorters'** (FACS) to afford a rather prompt and rapid sorting out of such characteristic cells.

4.5.1.5.4.5 Miscellaneous Applications

There are quite a few miscellaneous applications of MABs which would be discussed in the section that follows:

1. Drug Delivery and Targeting. The most vital and exemplory application of MABs in therapeutic domain is to precisely direct and guide a drug adequately conjugated with MABs against the neoplasm (tumour) antigens strategically positioned on the target cells. In other words, a **'toxic**

drug entity' is very selectively and precisely delivered to the target cell (*i.e.*, neoplasm cells) without causing any affect on the normal cells. Hence, in the latest therapeutic (pharmacologic) terminology such highly specific **drug-conjugated MAB** invariably act as **'Magic Bullets'.**

Advantages: The various advantages of **'Magic Bullets'** are as follows:

(*a*) The desired *'toxic-drug'* entity is solely prevented from circulation in the body, whereas a **'small dosage'** may be administered most precisely to the *'desired target'* in a rather effective manner; and

(*b*) The aforesaid technique is found to be an extremely useful one for the meticulous administration of *anti-neoplastic agent's viz.*, **methotrexate, busulfan, phosphoramide mustard, lomustine** and the like, without resulting into any serious side-effects whatsoever.

Notes: *The most challenging and difficult aspect of the entire exercise is the problem of raising MABs which shall get bound to* ***'neoplasm cells'*** *rather than the* ***'normal cells'****.*

2. Identification of Lymphocyte Subpopulations. A major break through and spectacular advancement in the application of MABs has been critically focused towards the identification for the sub-populations of lymphocytes by the help of **Fluorescence-Activated Cell Sorter** (FACS).

Methodology: The various steps involved are as follows:

(*a*) The fluorescent dye-labeled MABs absolutely specific for cell-surface antigenic determinants are made to interact with the ensuing lymphocytes.

(*b*) The resulting cells are subsequently passed through a *thin stream of culture medium via* an electric field.

(*c*) Consequently, the fluorescent cells pick-up charges accordingly; and, therefore, may be isolated from the non-flurescent ones conveniently.

Importance: The aforesaid technique is abundantly utilized not only to differentiate but also to segregate the fluorescent cells so as to pin-point effectively the prevalent and relative abundance of various kinds of **immune cells.** Therefore, the above delicate phenomenon may be exploited as a viable, dependable and trustworthy **'diagnostic tool'** for various **autoimmune diseases** and **immune deficiency disease conditions.**

3. Autoimmune and Immunodeficiency Diseases. It has been proved and well established beyond any reasonable doubt that in both *autoimmune* as well as *immune-deficiency diseases* the prevalent helper and suppressor T cell subset ratio gets disturbed appreciably. Therefore, the overwhelming T cell deficiencies may be monitored precisely and accurately by the aid of MABs-directed against the specific *T cell immunogens* (antigens).

4. Detection of Surface Molecules. MABs have been skillfully and purposefully employed to probe the **surface of immunocompetent cells.** Besides, MABs have also been used in *mapping* the actual prevailing *distribution of the membrane determinants, e.g., major histocompatibility complex* (MHC) *antigens* and a plethora of macromolecules. It is, however, a well known fact that the ensuing *T cell subsets* essentially comprise of various surface markers that are *predominantly antigenic in nature.*

In the therapeutic armamentarium the particular role and unique ability to *deplete a specific T cell subset* in a patient by the induction of MABs that have the capability and vulnerability to get bound selectively to one specific determinant of the aforesaid subset.

Example: The above critical situation may be further expatiated by specifically discarding the cytotoxic T cells in patients that might have been provided with a kidney transplantation from a donor, whereby the chances of *kidney rejection* (or *graft rejection*) in the **recipient** (*i.e.*, **new host**) **is minimized substantially.**

5. Veterinary and Plant Diagnostics. It has been duly recommended that the MABs could be employed extensively towards the diagnosis of Foot and Mouth disease in animals. They are also beneficial in the measurements of reproductive hormone levels in the animals. Interestingly, the

utilization of MABs in *plant viral diseases* is not so quite predominantly recognized as in humans or animals.

In nut shell, the above applications are representatives of the numerous applications of MABs the technological advancement of which is advancing in an astronomical speed as well as momentum.

4.6 Hypersensitivity Reactions

Hypersensitivity may be defined as – '*an abnormal sensitivity to a stimulus of any kind*'. Invariably, a situation may crop up when an *antigen* specifically interacts with a *sensitized* host thereby giving rise to tissue damage; and this is usually termed as **hypersensitivity reactions.**

In other words, one may dodge an explanation that immune reactions are particularly responsible for not only managing but also tackling adequately the invasion by a host of so called *'foreign antigens'* necessarily comprising of various types of viruses, microorganisms, chemicals, drug substances, allergens etc., and ultimately render reasonable protection to the human body as a whole. Therefore, a majority of prevailing *immune responses* are overwhelmingly useful for the human body; however, there are quite a few *immunological reactions* that significantly afford an almost **adverse reaction** ultimately resulting into undersirable, painful, and harmful effects. In true sense, these untowards hypersensitivity reactions are mostly characterized by a *highly specific antigen-antibody reaction (Ag-Ab reaction)*, and these are of *three* cardinal categories as **detailed below:**

i. When no visible reaction takes place even after the very first exposure to an '*agent*'. Evidently, the symptoms commence soon after *i.e.,* normally within a short span of a few days only after drug therapy begins,

ii. when the desired effects of an immunologic reaction utterly fail to resemble the pharmacological actions of the '*drug substance*' ; besides, such responses normally occur at dose levels much below the therapeutic limits (*i.e.,* the effective dose level), and

iii. When the prevailing immunologic reactions usually afford a restricted quantum of allergy related syndromes embracing only a small patient population.

There are ample evidences whereby certain therapeutic agents *i.e., 'drugs'*, quite often responsible for acute or severe adverse reactions (or allergic reactions), such as: penicillins, sulpha drugs, corticotrophin, erythromycin; besides several blood products. However, apparently most of these hypersensitive conditions *i.e.,* allergic reactions, may be observed on account of the '*inflammation*' occurring at the *very site of the Ag-Ab reaction*.

4.6.1 Types of Hypersensitivity Reaction

In actual practice, one may come across a variety of '*adverse reactions*' that may be conveniently categorized into the following *five* types, solely based on the prevailing underlying *immunologic mechanism,* namely:

(*a*) Type-I: Anaphylactic hypersensitivity,
(*b*) Type-II: Antibody-dependent cytotoxic hypersensitivity,
(*c*) Type-III: Complex mediated hypersensitivity,
(*d*) Type-IV: Cell-mediated or delayed type hypersensitivity and
(*e*) Type-V: Stimulatory hypersensitivity.

The five aforementioned types of hypersensitivity reactions ('*a*' through '*e*') shall now be dealt with individually in the section that follows:

4.6.1.1 Type-I: Anaphylactive Hypersensitivity

Anaphylactic hypersensitivity exclusively based upon the reaction of '*antigen*' with a particular IgE antibody intimately bond *via* its *crystallisable fragment* (Fc) to the corresponding *mast cell* there by leading to the ultimate release from the granules of the mediators '*histamine*', **slow**