Hybridoma Technology & Monoclonal Antibody Production

Definition:

Hybridoma A clone of hybrid cells formed by fusion of normal **lymphocytes** with **myeloma cells**. It retains the properties of the normal cell to produce antibodies or T-cell receptors but exhibits the immortal growth characteristic of myeloma cells. Hybridomas are used to produce **monoclonal antibody (mAB)**.

Monoclonal antibody Homogeneous preparation of antibody molecules, produced by a single clone of B lineage cells, often a hybridoma, all of which have the same antigenic specificity. (The term **monoclonal** refers to the fact that all of the cells in a given hybridoma culture are derived from the single clone of cells, and therefore carry the same DNA).

A hybridoma is a fusion product of two cells. B-cell hybridomas are generated by artificially fusing antibody-producing, short-lived lymphocytes with long-lived tumor cells in order to generate long-lived daughter cells secreting large amounts of monoclonal antibodies.

In 1975, Georges Köhler and Cesar Milstein figured out how to generate large quantities of antibodies derived from a single B-cell clone. By fusing a normal, activated, antibody-producing B cell with a myeloma cell (a cancerous plasma cell), they were able to generate a **hybridoma** that possessed the immortal growth properties of the myeloma cell parent and secreted the unique antibody produced by the B-cell parent. Over time, myeloma-cell partners were generated that had lost the ability to synthesize their own immunoglobulin, thus ensuring that the only antibodies secreted into the culture medium were those from the B-cell fusion partner. The importance of hybridomas to the biological sciences was recognized when Köhler and Milstein were awarded the Nobel Prize in Physiology or Medicine in 1984.

Process of Hybridoma production:

The original fusions used Sendai virus to disrupt the plasma membrane of the cells; nowadays, chemical **fusogens** such as **polyethylene glycol** (**PEG**) are used instead. In general, fusions between three or more cells are unstable, and the vast majority of fused cells growing out of these cultures are the products of the hybridization of two parent cells.

Fusion products:

1. Hybrids formed by the fusion of two antibody-producing B cells (B cells X B cells) will not grow out of these cultures because B cells have a relatively short half-life in vitro.
2. Hybrids formed by the fusion of B cells X myeloma- Resulting into production of B cell-myeloma hybrids is the target for **mAB** production.

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3. Hybrids formed by the fusion of two or more cancer cells (myeloma X myeloma) would have the potential to grow out from the initial fusions, and compete successfully for nutrients with the B cell-myeloma hybrids.

A method of selection of B cell-myeloma hybrids in HAT medium had been devised to eliminate these tumor-tumor hybrids from the cultures of fused cells.

HAT (medium named for its three components hypoxanthine, aminopterin and thymidine) **selection** depends on the fact that mammalian cells can synthesize nucleotide by two different pathways: the de novo & the salvage pathway. The de novo pathway, in which a methyl or formyl group is transferred from an activated form of tetrahydrofolate, is blocked by aminopterin, a folic acid analog. When the de novo pathway is blocked, cells utilize the salvage pathway, which bypasses the aminopterin block by converting purines & pyrimidines directly into nucleotides for synthesis of DNA & RNA. The enzymes catalyzing the salvage poathway include *hypoxanthine guanine phosphoribosyl transferase* (**HGPRT**) and *thymidine kinase* (**TK**). A mutation in either of these two enzymes blocks the ability of the cell to use the salvage pathway. HAT medium contains aminopterin to block the de novo pathway and hypoxanthine and thymidine to allow growth by the salvage pathway. Therefore cells that lack either HGPRT or TK will die in HAT medium. Because they lack the ability to use the salvage pathway to acquire essential intermediates for the synthesis of nucleic acids.

In hybridoma technology Köhler and Milstein solved this problem by using double mutant myeloma cells. They lack the enzyme *hypoxanthine guanine phosphoribosyl transferase* (HGPRT) and therefore are deselected in HAT medium. They have also lost the ability to produce immunoglobulin (Ig^- mutants). By using Ig^- mutants they assures that the antibodies produced by the hybridoma are encoded solely by the B cell partner and the myeloma cells only contribute immortal growth properties to the fused cells.

Köhler and Milstein reasoned that, if they grew the hybrid cultures in the presence of aminopterin, the mutant tumor cells (unfused) and tumor-tumor hybrids would be unable to synthesize new DNA by either the salvage or the de novo pathways and would eventually die. However, in the hybridomas formed by fusion between B cells and tumor cells (B cells X myeloma), the B-cell parent would provide the HGPRT for the salvage pathway, and so these hybrids would survive in the selection medium.

The resulting clones of hybridoma cells randomly lose chromosomes over the first few days following fusion, but eventually they stabilize and can be cultured indefinitely, secreting large quantities of mAbs of predefined specificity and known cross-reactivity.

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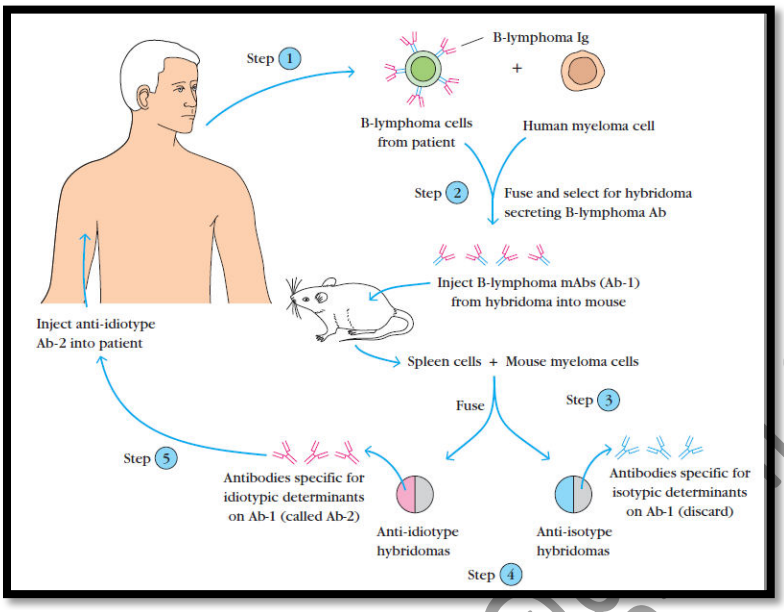

Fig: Development of a monoclonal antibody specific for idiotypic determinants on B-lymphoma cells.
Because all the B-lymphoma cells in a patient are derived from a single transformed B cell, they all express the same membranebound antibody (Ab-1) with the same idiotype (i.e., the same antigenic specifi city). In the procedure illustrated, a monoclonal anti-idiotypic antibody (Ab-2) against the B-lymphoma membrane-bound antibody is produced ex vivo (steps 1–4). This anti-idiotype antibody is then injected into the patient (step 5), where it binds selectively to the idiotypic determinants on the immunoglobulin of B-lymphoma cells, making these cells susceptible to complementmediated lysis.

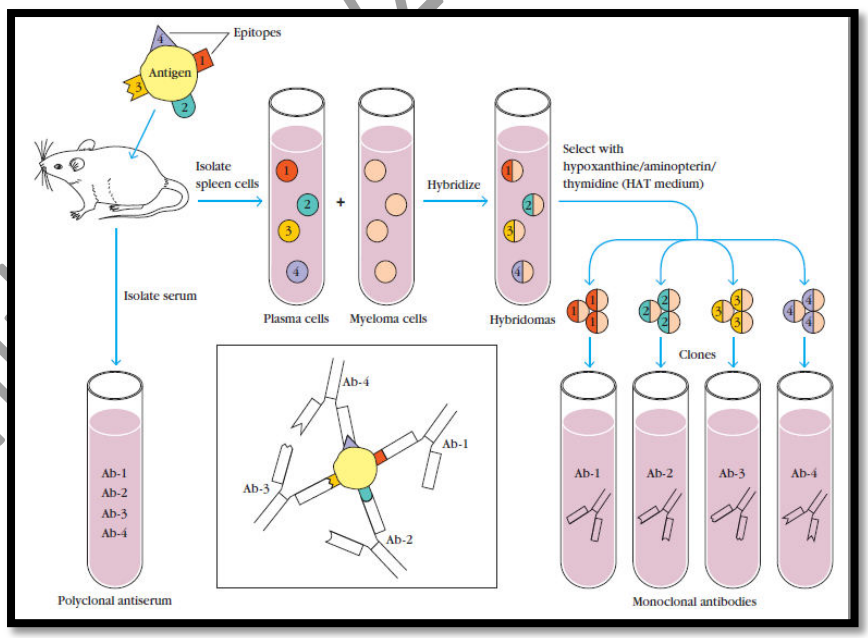

Fig: The conventional polyclonal antiserum produced in response to a complex antigen contains a mixture of monoclonal antibodies, each specific for one of the four epitopes shown on the antigen (inset). In contrast, a monoclonal antibody, which is derived from a single plasma cell, is specific for one epitope on a complex antigen. The outline of the basic method for obtaining a monoclonal antibody is illustrated here.

Monoclonal Antibody Production:

Once antibody secreting hybridomas are obtained, they are screened by RIA & ELISA techniques for the desired antigen specificity. Following identification, hybridomas are recloned to ensure that the culture is truly monoclonal and are then propagated in one of several ways to produce the desired monoclonal antibody. It can be grown in tissue culture flasks, mAb is secreted into the medium, at low concentration of 1-20ug/ml. A hybridoma can also be propagated in the peritoneal cavity of histocompatible mice, where it secretes mAb into the ascites fluid at much higher concentration of 1-10mg/ml. The antibody can be purified from the mouse ascites fluid by chromatography.

Clinical use for Monoclonal Antibodies:

Monoclonal antibodies are proving to be very useful as diagnostic, imaging, and therapeutic reagents in clinical medicine.

1. Among the many monoclonal antibody diagnostic reagents now available are products for – deetecting pregnancy, diagnosing numerous pathogenic microorganisms, measuring the blood levels of various drugs, matching histocompatibility antigens, and detecting antigens shed by certain tumors.
2. Radio-labeled monoclonal antibodies can be used in vivo for detecting or locating tumor antigens, permitting earlier diagnosis of some primary or metastatic tumors in patients.
3. Immunotoxins composed of tumor-specific monoclonal antibodies coupled to lethal toxins are potentially valuable reagents.
4. One novel use of mAbs has been the generation of antibodies that specifically bind and stabilize the transition state of a chemical reaction, thus directly mimicking the activity of enzymes. Such antibodies with enzyme-like activities are referred to as **abzymes**.

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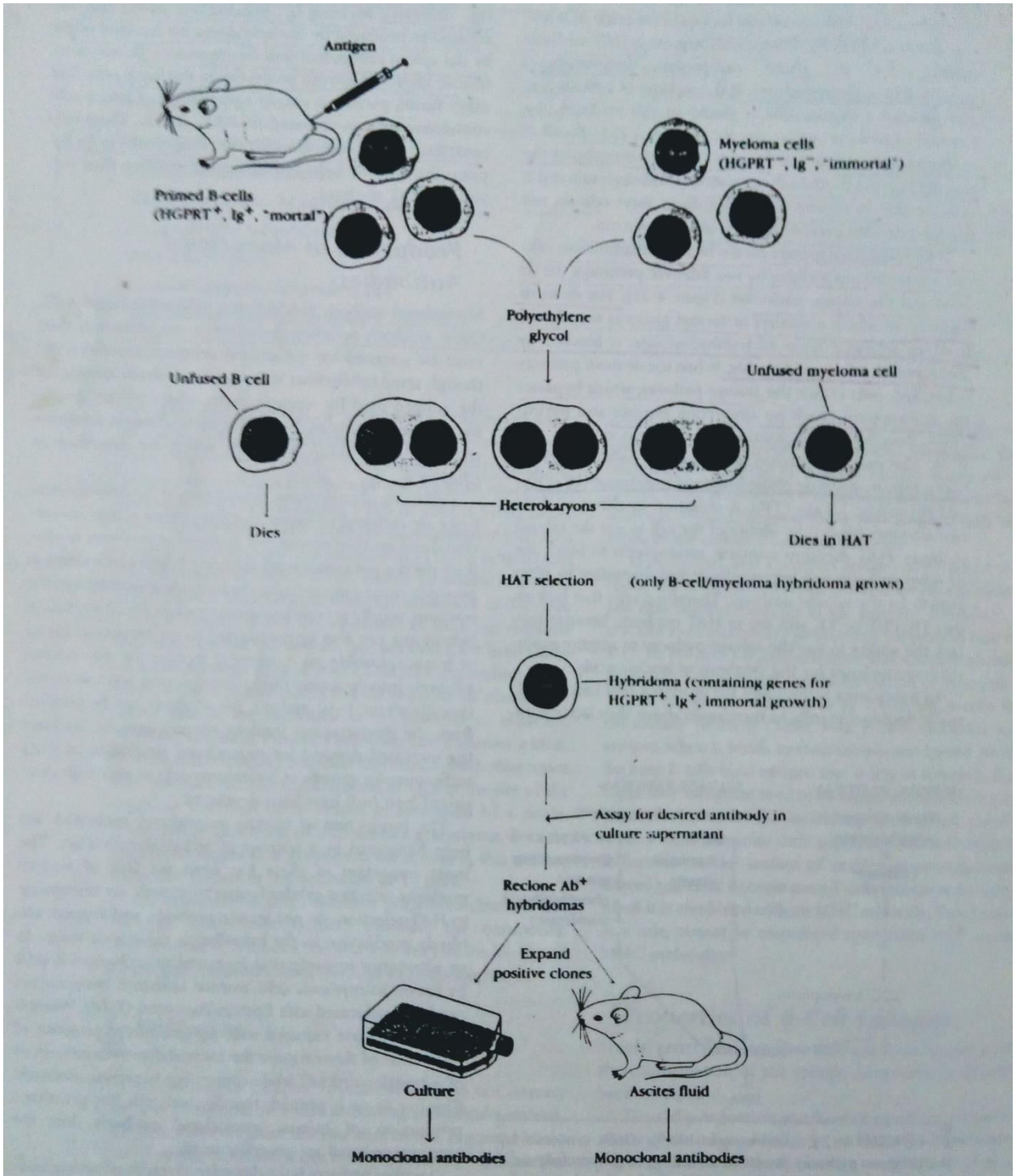

FIGURE 4-22 The procedure for producing monoclonal antibodies specific for a given antigen developed by G. Kohler and C. Milstein. Spleen cells (HGPRT$^+$ and Ig$^+$) from an antigen-primed mouse are fused with mouse myeloma cells (HGPRT$^-$ and Ig$^-$). The spleen cell provides the necessary enzymes for growth on HAT medium, while the myeloma cell provides immortal-growth properties. Unfused myeloma cells or myeloma/myeloma fusions fail to grow because of their lack of HGPRT. Unfused spleen cells have limited growth capabilities in vitro and will die within a few days.