Monoclonal antibody production

Monoclonal Antibodies

A tumor of plasma cells (myeloma or plasmacytoma) is monoclonal and therefore produces antibodies of a single specificity. In most cases, the specificity of the tumor-derived antibody is not known, so the antibody cannot be used to specifically detect or bind to molecules of interest. However, the discovery of monoclonal antibodies produced by these tumors led to the idea that it may be possible to produce similar monoclonal antibodies of any desired specificity by immortalizing individual antibody-secreting cells from an animal immunized with a known antigen. A technique to accomplish this was described by Georges Kohler and Cesar Milstein in 1975 and has proved to be one of the most valuable advances in all of scientific research and clinical medicine. The method relies on fusing B cells from an immunized animal (typically a mouse) with a myeloma cell line and growing the cells under conditions in which the unfused normal and tumor cells cannot survive (Fig. 5-9). The resultant fused cells that grow out are called hybridomas; each hybridoma makes only one Ig. The antibodies secreted by many hybridoma clones are screened for binding to the antigen of interest, and this single clone with the desired specificity is selected and expanded. The products of these individual clones are **monoclonal antibodies** that are each specific for a single epitope on the antigen or antigen mixture used to identify antibody-secreting clones.

Monoclonal antibodies have many practical applications in research and in medical diagnosis and therapy. Some of their common applications include the following:

- Identification of phenotypic markers unique to particular cell types. The basis for the modern classification of lymphocytes and other leukocytes is the recognition of individual cell populations by specific monoclonal antibodies. These antibodies have been used to define clusters of differentiation (CD) markers for various cell types (see Chapter 2).
- Immunodiagnosis. The diagnosis of many infectious and systemic diseases relies on the detection of particular antigens or antibodies in the circulation or in tissues by use of monoclonal antibodies in immunoassays (see Appendix IV).
- Tumor detection. Tumor-specific monoclonal antibodies are used for detection of tumors by imaging techniques and by staining tissues with labeled antibodies.
- Therapy. Advances in medical research have led to the identification of cells and molecules that are involved in the pathogenesis of many diseases. Monoclonal antibodies, because of their exquisite specificity, provide a means of targeting these cells and molecules. A number of monoclonal antibodies are used therapeutically today (Table 5-3). Some examples include antibodies against the cytokine tumor necrosis factor (TNF) used to treat rheumatoid arthritis and other inflammatory diseases, antibodies against CD20 for the treatment of B cell leukemias and for depleting B cells in certain autoimmune disorders, antibodies against the type 2 epidermal growth factor receptor to target breast cancer cells, antibodies against vascular endothelial growth factor (a cytokine that promotes angiogenesis) in patients with colon cancer, and so on.
- Functional analysis of cell surface and secreted molecules. In biologic research, monoclonal antibodies that bind to cell surface molecules and either stimulate or inhibit particular cellular functions are invaluable tools for defining the functions of surface molecules, including receptors for antigens. Monoclonal antibodies are also widely used to purify selected cell populations from complex mixtures to facilitate the study of the properties and functions of these cells.

TABLE 5–3 Monoclonal Antibodies of Therapeutic Significance

Target	Effect	Diseases
CD20	B cell depletion	Rheumatoid arthritis, multiple sclerosis, other autoimmune diseases
VEGF	Blocking of tumor angiogenesis	Breast cancer, colon cancer
HER2/Neu	Depletion of tumor cells with HER2 amplification	Breast cancer
TNF	Inhibition of T cell–mediated inflammation	Rheumatoid arthritis, Crohn's disease

One of the limitations of monoclonal antibodies for therapy is that these antibodies are most easily produced by immunizing mice, but patients treated with mouse monoclonal antibodies may make antibodies against the mouse Ig, called a human anti-mouse antibody (HAMA) response. These anti-Ig antibodies eliminate the injected monoclonal antibody and can also cause serum sickness. Genetic engineering techniques have been used to expand the usefulness of monoclonal antibodies. The complementary DNAs (cDNAs) that encode the polypeptide chains of a monoclonal antibody can be isolated from a hybridoma, and these genes can be manipulated in vitro. As discussed before, only small portions of the antibody molecule are responsible for binding to antigen; the remainder of the antibody molecule can be thought of as a framework. This structural organization allows the DNA segments encoding the antigen-binding sites from a mouse monoclonal antibody to be "stitched" into a cDNA encoding a human myeloma protein, creating a hybrid gene. When it is expressed, the resultant hybrid protein, which retains the antigen specificity of the original mouse monoclonal but has the core structure of a human Ig, is referred to as a humanized antibody. Humanized antibodies are far less likely than mouse monoclonals to appear "foreign" in humans and to induce anti-antibody responses.

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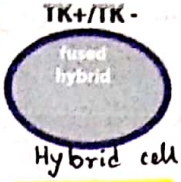
HAT Selection

Myeloma cell
- Immortal
- Unable to secrete immunoglobulins

Splenic B-cell
- limited life span.
- Can secrete immunoglobulins

	HGPRT⁻ TK-	HGPRT⁺/HGPRT⁻ TK+/TK-	HGPRT⁺ TK+
Genotype:*			
Cell type:	 Myeloma cell	 Hybrid cell	 B-cell
HAT fate:	DIES	SURVIVES	DIES
Explanation:	**Unable to synthesize DNA:** (1) Thymidine kinase* mutation causes a loss-of-function in the "salvage" pathway and (2) Aminopterin blocks "De novo" pathway.	**Immortal and restored DNA synthesis:** (1) Immortality from plasmacytoma and (2) rescued ability to synthesize DNA due to restored thymidine kinase* function.	**Mortal:** (1) Functional DNA synthesis, but (2) eventually dies because of limited number of replication cycles

*HGPRT (hypoxanthine-guanine phosphoribosyltransferase) mutants can be used in place of TK (thymidine kinase) mutants

De novo synthesis:	✗	✗	✗
Salvage pathway:	✗	✓	✓

Monoclonal antibody		Polyclonal antibody
Monoclonal antibody refers as an antibody of single specificity, secreted from the single clone of antibody producing immortalized hybrid cells in vitro.	Definition	Polyclonal antibodies refer to a mixture of heterogeneous population of immunoglobulin molecules that are secreted against a particular antigen with multiple epitopes.
Recognizes only one epitope on an antigen.	Epitope	Recognizes multiple epitopes on any one antigen.
Expensive to produce.	Expense	Inexpensive to produce.
High technology is required.	Technology required	Technology required is low
High training skill is required for the technology use.	Skill required	Low training skill is required.
Used as therapeutic drugs.	Application	Used as general research purpose.
Possesses less cross reactivity.	Cross-reactivity	Possesses comparatively high cross-reactivity.

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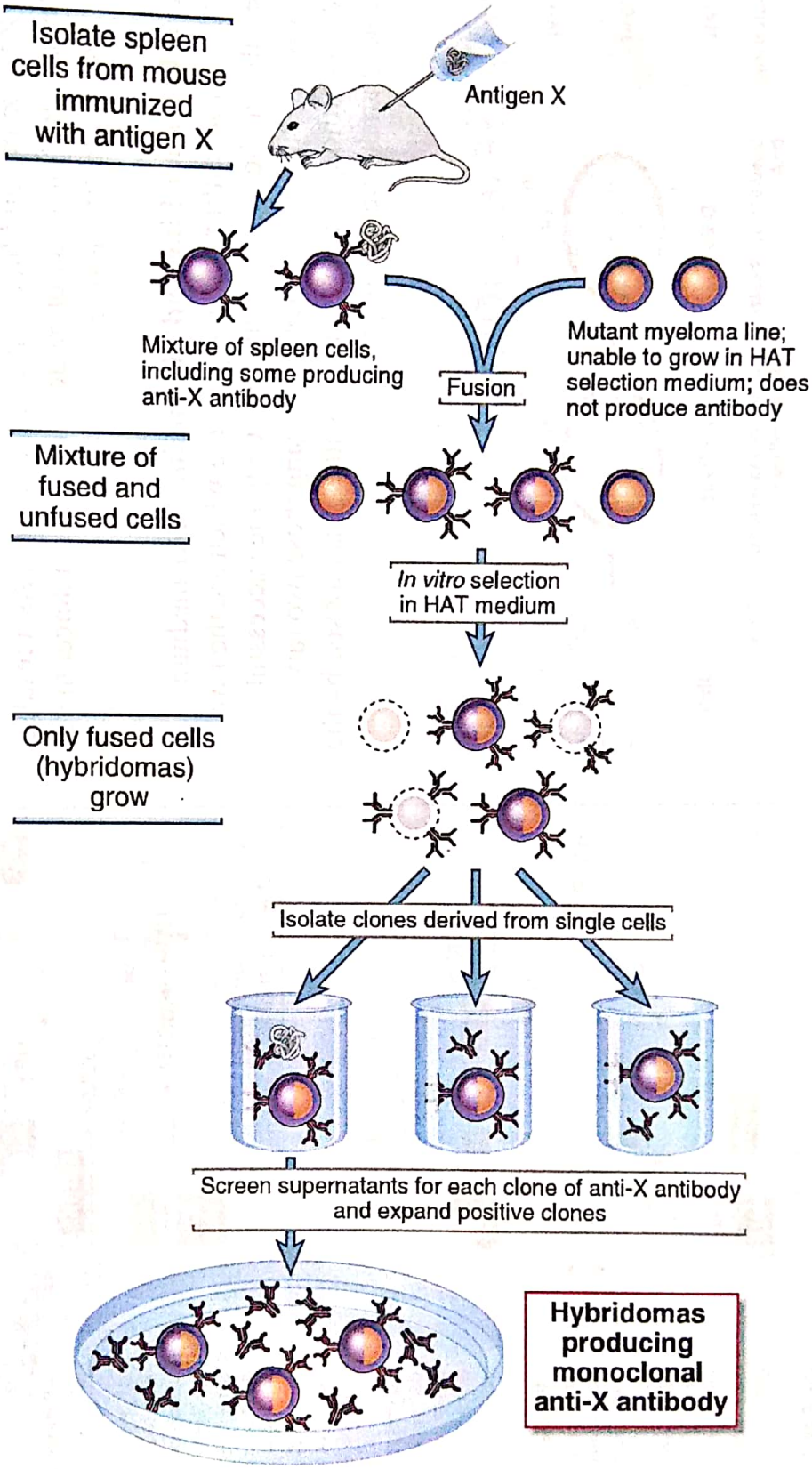

FIGURE [redacted] The generation of monoclonal antibodies. In this procedure, spleen cells from a mouse that has been immunized with a known antigen or mixture of antigens are fused with an enzyme-deficient partner myeloma cell line, with use of chemicals such as polyethylene glycol that can facilitate the fusion of plasma membranes and the formation of hybrid cells that retain many chromosomes from both fusion partners. The myeloma partner used is one that does not secrete its own Igs. These hybrid cells are then placed in a selection medium that permits the survival of only immortalized hybrids; these hybrid cells are then grown as single cell clones and tested for the secretion of the antibody of interest. The selection medium includes hypoxanthine, aminopterin, and thymidine and is therefore called HAT medium. There are two pathways of purine synthesis in most cells, a de novo pathway that needs tetrahydrofolate and a salvage pathway that uses the enzyme hypoxanthine-guanine phosphoribosyltransferase (HGPRT). Myeloma cells that lack HGPRT are used as fusion partners, and they normally survive using de novo purine synthesis. In the presence of aminopterin, tetrahydrofolate is not made, resulting in a defect in de novo purine synthesis and also a specific defect in pyrimidine biosynthesis, namely, in generating TMP from dUMP. Hybrid cells receive HGPRT from the splenocytes and have the capacity for uncontrolled proliferation from the myeloma partner; if they are given hypoxanthine and thymidine, these cells can make DNA in the absence of tetrahydrofolate. As a result, only hybrid cells survive in HAT medium.