Radioimmunotherapy (RIT) 9

9.1 Introduction

For the purposes of this discussion, radioisotopes are attached to antibodies for either diagnostic or therapeutic application. The use of antibodies radiolabeled with therapeutic radioisotopes is referred to as radioimmunotherapy (RIT). This technology has evolved from the spectacular advances and growth in the fields of molecular biology and biotechnological. The ability of an antibody to recognize the cognate target antigen with exquisite specificity is the genesis of RIT. In particular, the use of monoclonal antibodies (mAb) radiolabeled with therapeutic radionuclides directed against specific tumor antigens is a unique strategy for site-specific delivery of radiation directly to the tumor, and this represents the cornerstone for the success of RIT. In this context, the invention of hybridoma technology by Kohler and Milstein in 1975 merits special attention (Kohler and Milstein 1975). In 1984, they shared the Nobel Prize for Physiology and Medicine just 9 years after publishing their seminal paper on hybridoma technology. The pioneer research of Kohler and Milstein resulted for the first time in the production of rodent antibodies of single specificity (monoclonal antibodies), and this triggered the use of monoclonal antibodies as delivery vehicles for radionuclides for therapy. RIT combines the synergistic effects of both radiation and immunotherapy with manageable local and systemic side effects. The characteristic and complex interactions between the tumor, host, radionuclide, and the antigen–antibody complex determine the effectiveness of RIT.

9.2 Identification of Cell Surface Markers

The cluster of differentiation (CD) is a protocol used for the identification and investigation of cell surface molecules present on leukocytes. CD molecules can act in numerous ways, often acting as receptors or ligands (the molecule that activates a receptor) important to the cell. The CD nomenclature was proposed and established in the 1st International Workshop and Conference on Human Leukocyte Differentiation Antigens (HLDA) (Zola et al. 2005; Zola and Swart 2005). This system was intended for the classification of the many monoclonal antibodies (mAbs) generated by different laboratories around the world against epitopes on the surface molecules of leukocytes (white blood cells). Since then, its use has expanded to many other cell types, and more than 320 CD unique clusters and subclusters have been identified. The human leukocyte antigen (HLA) is expressed as cell surface molecules/antigens on various immune cells. Through flow cytometry, the CD markers or HLA molecules are identified using mAbs directed against a specific cell surface antigen. Under the current naming system, antigens that are well characterized are assigned an arbitrary number (e.g., CD1,

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CD2, etc.), whereas molecules that are recognized by just one monoclonal antibody are given the provisional designation "CDw." Physiologically, CD antigens do not belong in any particular class of molecules, with their functions ranging from cell surface receptions to adhesion molecules. Although initially used for just human leukocytes, the CD molecule naming convention has now been expanded to cover both other species (e.g., mouse) as well as other cell types. Human CD antigens are currently numbered up to CD363. Before discussing RIT in detail, it is helpful to provide a brief introductory discussion on antibodies, antigens, and lymphoma and their roles in RIT.

9.3 Antibodies

Antibodies are immune system-related proteins called immunoglobulins. A typical antibody molecule is made up of four polypeptide chains. The four chains are divided into two identical light chains and two identical heavy chains (Fig. 9.1). An antibody molecule is depicted as Y-shaped molecules with two identical antigen-binding sites at the ends of the arms of the Y. The light and heavy chains contribute to the antigen-binding sites to receptors or specific "antigens." Each antibody molecule can bind to two identical antigenic determinants. Where the arms meet, the stem of the Y is known as the hinge region. The hinge region allows segmental flexibility of the antibody molecule. The two antigen-binding ends (or amino-terminal ends) of the antibody molecule are called the Fab fragments (for fragment antigen binding), whereas the stem (or carboxyl-terminal end) of the Y is considered to be the Fc fragment (for fragment crystallizable). The Fc region of the antibody molecule is responsible for its biologic properties.

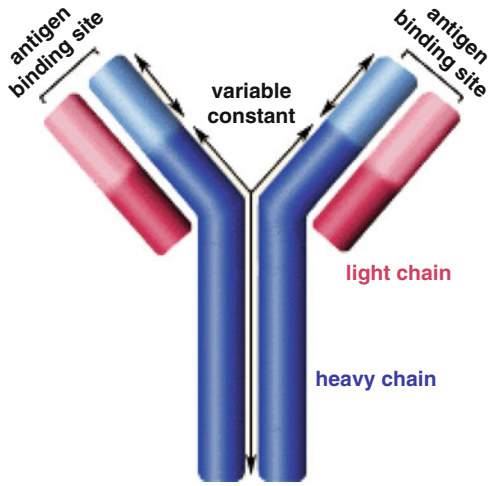

Fig. 9.1 Illustration of antibody structural components

The amino-terminal end of an antibody is called the variable, or V, region and the carboxyl-terminal end is called the constant, or C, region. The C region is about the same size as the V region in the light chain and three to four times larger than the V region in the heavy chain. The V regions of light and heavy chains form the antigen-binding sites. Light and heavy chains consist of repeating, similarly folded homology units or domains. The light chain has one V region (VL) domain and one C region (CL) domain, whereas the heavy chain has one V region (VH) and three or four C region (CH) domains. The most variable parts of the V regions are limited to several small hypervariable regions or complementarity-determining regions (CDRs). The light- and heavy-chain V regions contain three CDRs. The CDRs come together at the amino-terminal end of the antibody molecule to form the antigen-binding site, which determines specificity. The invariant regions of amino acids, between the CDRs, make up about 85 % of the V regions and are designated framework residues. The three functions which explain antibody chemical structure include binding versatility, binding specificity, and biological activity.

9.3.1 B Cells and T Cells

White blood cells (lymphocytes) play an important role in the immune response. While the production of lymphocytes is initiated in bone marrow, after maturation, these circulate through the blood and lymphatic vessels. Lymphocytes fall into the B-cell and the T-cell groups, and each cell is specific for a particular antigen which

resides in a receptor for antigen including the B-cell receptor (BCR) for antigen and the T-cell receptor (TCR), respectively. Both BCRs and TCRs are integral membrane proteins displayed on their surface and present in thousands of identical copies exposed at the cell surface, available even before the cell ever encounters an antigen and encoded by genes assembled by the recombination of segments of DNA. However, they differ in structure and the genes that encode them and the type of epitope to which they bind.

Different B cells carry different antibodies, and when a foreign organism invades through the bloodstream, it encounters B cells which express antibodies that can recognize one of the invading antigens. Subsequently, a concerted action follows involving T cells and cells which are called "phagocytes," where binding of the antigen to the B cell causes further rapid and repeated B-cell division. The resulting collection of daughter cells is called a "clone." The T cells provide several activities collectively referred to as "cellular immunity," which is an especially important capability of the body to fight against viruses and to assist B cells for antibody production. The T cells have surface membrane protein receptors which recognize and bind to specific antigens. However, this only occurs when the antigens are attached on cell surfaces. Once the T cells have bound to the antigen, they divide quickly and repeatedly to form an activated clone (Tosato et al. 1980).

9.3.2 Polyclonal and Monoclonal Antibodies

Polyclonal antibody mixtures contain different antibodies developed in the blood of immunized animals from different cell types. Polyclonal antibodies are a combination of immunoglobulin (IgG) molecules secreted against a specific antigen, each identifying a different epitope. As most antigens bear multiple epitopes, they can stimulate the proliferation and differentiation of a variety of B-cell clones. Thus, a heterogeneous pool of serum antibodies can be produced with specificity for particular epitope(s) of the antigen. Polyclonal antibodies recognize multiple epitopes on any one antigen, and the serum obtained will contain a heterogenous complex mixture of antibodies of different affinities. Polyclonals will recognize multiple epitopes on any one antigen and this process has several advantages which include high affinity, since polyclonals can assist in signal amplification from target protein with low expression level, since the target protein will bind more than one antibody molecule on the multiple epitopes. Polyclonal antibody mixtures react with multiple epitopes on the surface of the antigen and will thus be more tolerant of minor changes in the antigen, e.g., polymorphism, heterogeneity of glycosylation, or slight denaturation, than will monoclonal (homogenous) antibodies. Due to recognition of multiple epitopes, polyclonals can provide better results in IP/ChIP and will identify proteins of high homology to the immunogen protein or can be used to screen for the target protein in tissue samples from species other than that of the immunogen. This is because polyclonal antibodies are sometimes used when the nature of the antigen in an untested species is unknown. This capability also makes it important to check immunogen sequence for any cross-reactivity. Often, polyclonal antibodies are the preferred choice for detection of denatured proteins, since multiple epitopes generally provide more robust detection.

9.3.3 Monoclonal Antibodies (mAbs)

In contrast, monoclonal antibodies (mAbs or moAbs) are a mixture of homogenous antibody molecules with affinity towards a specific antigen as they are made by identical immune cells that are several copies of a same parent cell, often generated using a hybridoma by fusing a B cell with a single lineage of cells containing a definite antibody gene (Fig. 9.2).

The availability of mAb through hybridoma technology had revolutionized immunology and has had far reaching implications for a large number of technologies. Monoclonal antibodies have monovalent affinity and homogeneous, in that they bind to the same epitope which makes them effective in therapies. The mAb consists of

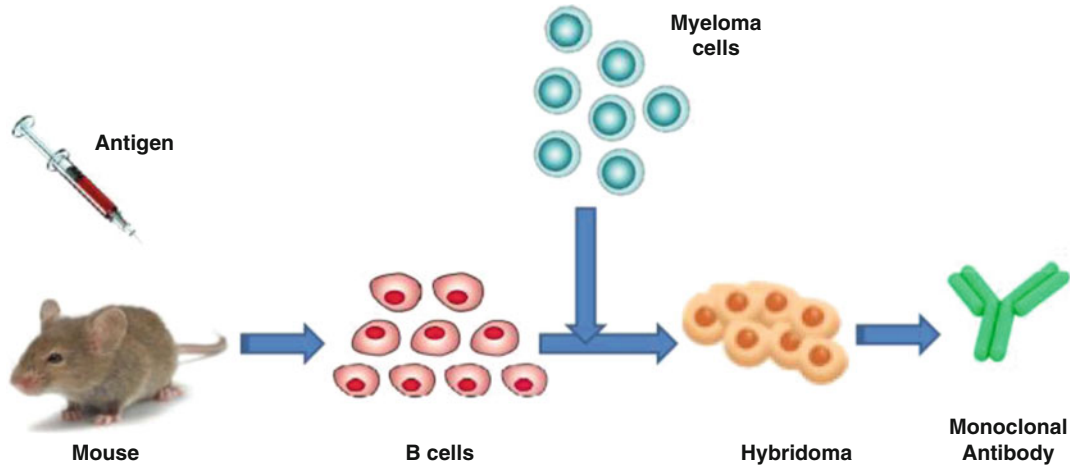

Fig. 9.2 Production of a monoclonal antibody through hybridoma fusion of a B-cell with a single lineage of cells

only one antibody subtype (e.g., IgG1, IgG2, IgG3) that has high specificity, i.e., detects only one epitope on the antigen. As they are more specifically detecting one target epitope, they are less likely to cross-react with other proteins. Compared to polyclonal antibodies, homogeneity of monoclonal antibodies is very high. Specificity of monoclonal antibodies makes them extremely efficient for binding of antigen within a mixture of related molecules. The mAbs are more vulnerable to the loss of epitope through chemical treatment of the antigen than polyclonal antibodies. The basic technique involved in making of monoclonal antibodies relies on fusion of B cells from an immunized mouse with a myeloma (tumor cell line) cell line and let the cells grow in a condition where unfused normal and tumor cells cannot survive. The cells that are fused and able to grow through this procedure are called as hybridomas. Table 9.1 illustrates the key differences between polyclonal and monoclonal antibodies.

Table 9.1 Key differences between polyclonal and monoclonal antibodies

Polyclonal antibodies	Monoclonal antibodies
Inexpensive to produce	Expensive to produce
Skills required are low	Training is required for the technology used
Time scale is short	Time scale is long for hybridomas
Produces large amounts of nonspecific antibodies	Can produce large amounts of specific antibodies
Recognizes multiple epitopes on any one antigen	Recognizes only one epitope on an antigen
Can have batch-to-batch variability	No or low batch-to-batch variability
	Once a hybridoma is made, it is a constant and renewable source

The success of RIT largely depends on the production of sufficient amounts of monoclonal antibodies which are required for therapy. Table 9.2 provides information for various key antibodies developed for RIT (Fig. 9.3).

The majority of early clinical studies using radioimmunoconjugates—where therapeutic radioisotopes are attached to antibodies discussed later in—failed to demonstrate a therapeutic impact because monoclonal antibodies of murine origin had been used which are immunogenic in humans and thus prevent repeated administration to patients. Additionally, most radioimmunotherapy approaches for the treatment of solid tumors have proven ineffective because the radiation dose delivered to neoplastic masses was insufficient to induce objective responses and cures. In more recent years, the limitation imposed by the use of monoclonal antibodies of murine origin has been circumvented by the availability and use of more refined chimeric, humanized, and fully

human antibodies that can be mass produced (Winter and Harris 1993). The use of techniques to humanize or chimarize monoclonal antibodies to decrease their murine components has been an important advance in this field. These molecules have a long vascular half-life and can interact with human complement or effector cells of the patient immune system. These molecules also behave in a manner similar to naturally occurring immunoglobulin and work along the lines of our normal antibody-based immune response as effective agents in treating patients with cancer (Dillman 2003). Table 9.3 provides information describing a variety of commercially available humanized or chimarized monoclonal antibodies and used in various clinical indications.

Table 9.2 Examples of antibodies developed for RIT

Antibody designation	Antigen designation	Type of antibody
Lym-1	HLA-DR10	Murine IgG2a
anti-B1	CD20	Murine IgG2a
2B8	CD20	Murine IgG1
C2B8	CD20	Chimeric IgG1
hLL2	CD22	Humanized IgG1
MB-1	CD37	Murine IgG1
Campath-1H	CD52	Humanized IgG

Abbreviations: *CD* acronym for the "cluster of designation", *IgG* immunoglobulin G

9.4 Antigens

An antigen is defined as "any foreign substance that elicits an immune response (e.g., the production of specific antibody molecules) when introduced into the tissues of a susceptible animal and is capable of combining with the specific antibodies formed." Antigens are generally of high molecular weight and are commonly proteins, carbohydrates, or polysaccharides, lipids, nucleic acids, or even small molecules like neurotransmitters which can act as antigens. These molecules elicit an immune response involving the production of specific antibody molecules when introduced into the tissues of a susceptible animal or human. The antigens are capable of combining with the specific antibodies which are formed. Specific antibodies can interact with

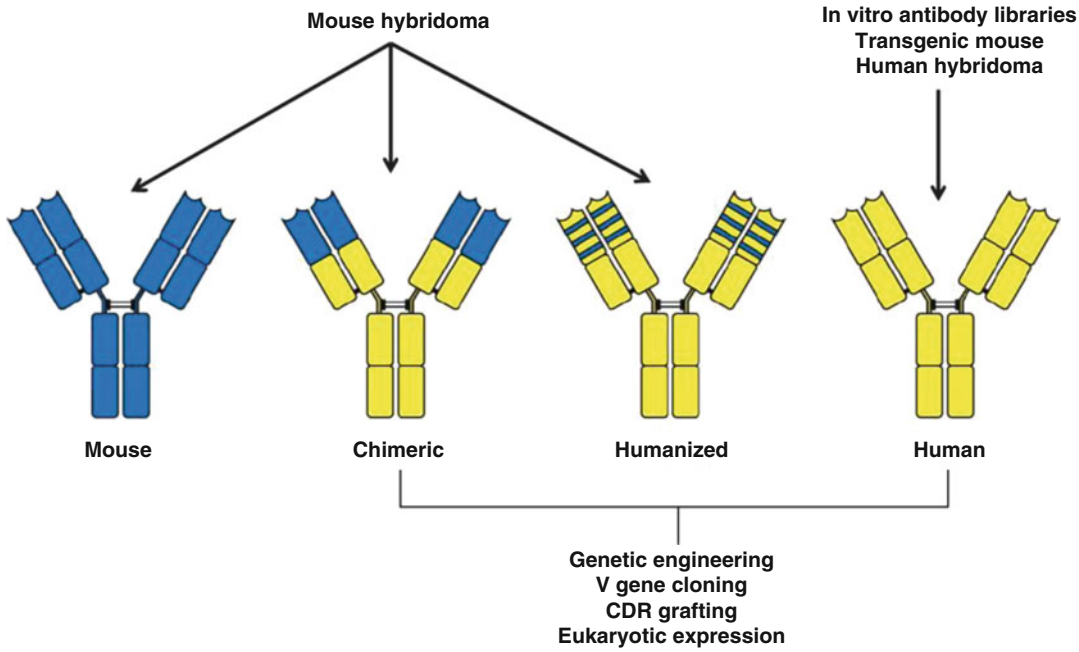

Fig. 9.3 Examples of antibody engineering strategies to obtain "humanized" antibodies

Table 9.3 Key examples of commercially available monoclonal antibodies

Monoclonal antibody	Target antigen	Clinical application
OKT3	CD3 antigens on T lymphocytes	Acute rejection of transplanted kidney, heart, and liver
Abciximab GP	IIb/IIIa on platelets	Antithrombotic applications
Rituximab	CD20 receptors on B lymphocytes	Non-Hodgkin's lymphoma
Daclizumab	Interleukin-2 receptors on activated T lymphocytes	Acute rejection of transplanted kidneys
Trastuzumab (Herceptin)	HER-2 growth factor receptors	Advanced breast carcinomas expressing HER-2 receptors
Infliximab	TNF (tumor necrosis factor)	Rheumatoid arthritis and Crohn's disease
Basiliximab	Interleukin-2 receptors on activated T lymphocytes	Acute rejection of transplanted kidney
Palivizumab	F protein of respiratory syncytial virus (RSV)	RSV infection in children
Gemtuzumab	CD33 antigen	Relapsed acute myeloid leukemia
Alemtuzumab	CD52 antigen on B and T lymphocytes	B-cell chronic lymphocytic leukemia
Cetuximab	EGFR (epidermal growth factor receptor)	Colorectal carcinoma and some other tumors

Abbreviations: *NHL* non-Hodgkin's lymphoma, *VEGF* vascular endothelial growth factor, *EGFR* epidermal growth factor receptor

only a small region of an antigen, and in the case of a polypeptide, this is generally about 5–12 amino acids. This region can be continuous or it can be distributed in different regions of a primary structure that are brought together because of the secondary or tertiary structure of the antigen. The antigen region which is recognized by an antibody is called an "epitope" and usually consists of one to six monosaccharides or 5–8 amino acid residues on the antigen surface. Because antigen molecules exist in space, the epitope recognized by an antibody may be dependent upon the presence of a specific three-dimensional antigenic conformation. This may be represented by a unique site formed by the interaction of two native protein loops or subunits. In addition, the epitope may correspond to a simple primary sequence region. The range of possible binding sites is enormous, with each potential binding site exhibiting its own structural properties derived from covalent bonds, ionic bonds, and hydrophilic and hydrophobic interactions. For efficient interaction to occur between the antigen and the antibody, the epitope must be readily available for binding. If the target molecule is denatured, e.g., through fixation, reduction, pH changes, or during preparation for gel electrophoresis, the epitope may be altered, and this may affect its ability to interact with an antibody. The epitope may be present in the antigen's native, cellular environment, or only exposed when denatured. In their natural form, they may be cytoplasmic (soluble), membrane associated, or secreted. The number, location, and size of the epitopes depend on how much of the antigen is presented during the antibody-making process.

There are a large variety of important characteristics of biologic antigens. The most striking feature of antigen–antibody interactions is the high specificity and affinity. It is accepted that almost all the antigens are identified by specific antibodies, but very few have the ability to stimulate the antibodies. Often, it is useful to illicit the formation of antibodies in response to small molecules of interest. However, in contrast to macromolecular molecules which can often illicit the formation of antibodies, small molecules cannot independently provoke an immune response and thus antibody formation. To overcome this, immunologists can attach several copies of small

molecules of interest, called "haptens," to a carrier protein prior to immunization. In this manner, the antibodies which are formed are specific to the hapten/carrier–protein complex. Each antibody binds to a particular part of the antigen called the antigenic determinant (or epitope), which is the specific site on an antigen to which the specific antibody binds. Epitopes are hence also called as antigenic determinants. The random structure on the antigenic molecule that are identified by the antibody as an antigenic binding site thus form the epitope of that antigen. The strength of the antibody–antigen binding is important, and the binding strength between an antigenic determinant in an antigen (epitope) and an antigen-binding site in an antibody (paratope) is referred to as the affinity. Different epitopes can be organized on a single protein molecule in such a manner that their spacing may affect the binding of antibody molecules in various ways. There are a variety of specific terms used in RIT which include the following.

9.4.1 Affinity

This term describes the strength of binding between one antibody-binding site and an antigenic determinant (epitope or hapten). It is the sum of the attractive and repulsive forces, which include van der Waals interactions, hydrogen bonds, salt bridges, and hydrophobic force, although the exact contribution of each of these factors depends on the particular antigen–antibody pair and the combining site of the antibody. The affinity is thus the equilibrium constant that describes the antigen–antibody reaction. The potency of the reaction between a specific antigenic determinant and its single combining site on the antibody determines its affinity. Most antibodies have a high affinity for their antigens.

9.4.2 Avidity

The strength with which a multivalent antibody binds a multivalent antigen is termed avidity, to differentiate it from the affinity of the bond between a single antigenic determinant and an individual combining site. The avidity of an antibody for its antigen is determined by the sum of all of the individual interactions taking place between individual antigen-binding sites of antibodies and determinants on the antigens. The avidity of an antibody for its antigen strongly depends on the affinities of the individual combining sites for the determinants on the antigens. It is controlled by three major factors: antibody epitope affinity, the valence of both the antigen and antibody, and the structural arrangement of the interacting parts. Avidity is more than the sum of the individual affinities.

9.4.3 Specificity

Specificity refers to the ability of an individual antibody-combining site to react with only one antigenic determinant or the ability of a population of antibody molecules to react with only one antigen. In general, there is a high degree of specificity in antigen–antibody reactions. Antibodies can distinguish differences in the primary structure of an antigen, isomeric forms of an antigen, and secondary and tertiary structure of an antigen.

9.4.4 Cross-Reactivity

This term refers to the ability of an individual antibody-combining site to react with more than one antigenic determinant or the ability of a population of antibody molecules to react with more than one antigen. Cross-reactions arise because the cross-reacting antigen shares an epitope in common with the immunizing antigen or because it has an epitope which is structurally similar to one on the immunizing antigen (multispecificity). Normally, antigen–antibody-binding site on antibodies is essentially flat and hence spacious so that they can attach large complexes or structures (Fig. 9.4).

9.5 Lymphomas

Lymphomas are malignancies of the lymphoid tissue and are broadly classified into Hodgkin's lymphoma (HD) and non-Hodgkin's

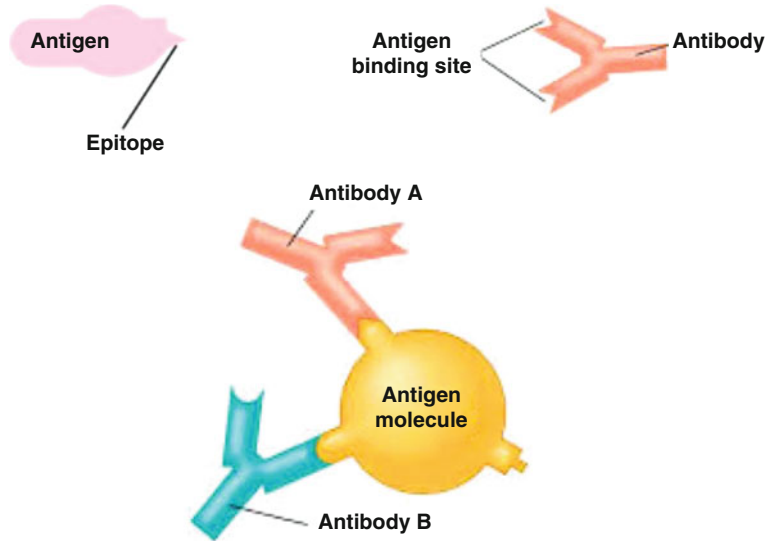

Fig. 9.4 Antibody–antigen binding depends on both specificity and avidity

lymphomas (NHL, 85 %). The hallmark of Hodgkin's lymphoma (HL) is the presence of large, mononucleated Hodgkin and multinucleated Reed/Sternberg cells. These cells represent the tumor cells, but usually comprise less than 1 % of the cellular infiltrate in the lymphoma tissue (Weiss et al. 1999). Due to the rarity of the Hodgkin and Reed/Sternberg (HRS) cells and their unusual phenotype, the origin of these cells from germinal center (GC) B cells in both the lymphocyte predominant (LP) and the classical subtype of HL has only recently been clarified (Küppers 2002). Only in very rare cases do the HRS cells of classical HL represent transformed T cells (Müschen et al. 2000; Seitz et al. 2000).

In contrast, non-Hodgkin's lymphomas are a heterogeneous group of lymphoreticular malignancies with a wide range of aggressiveness. The majority of NHL are B-cell lymphomas, with the follicular and diffuse large B-cell lymphomas constituting up to 50 % of NHL. NHL can also be classified as indolent (i.e., slow progression; 40 %) or aggressive lymphomas (60 %). B-cell CLL/small lymphocytic lymphoma, marginal zone lymphoma, lymphoplasmacytoid, and follicle center lymphoma constitute the indolent types, whereas diffuse large B-cell, mantle cell, Burkitt's, and precursor B-cell leukemia constitute the aggressive types. NHL accounts for 4 % of all malignancies and 4 % of all cancer-related deaths (Anand et al. 2008).

9.6 Radioimmunotherapy (RIT)

The preceding discussions represent the foundation required to describe the use of antibodies radiolabeled with therapeutic radioisotopes for RIT. Radioimmunotherapy (RIT) uses monoclonal antibodies as the vector for transport of the radioactivity to cancer cells (Chatal and Mahé 1998). Thus, the antibody–antigen-binding targeting mechanism and the radioisotope which decays by emission of ionizing radiation is the payload. The radiolabeled antibodies are directed against various antigens overexpressed on tumor cells or blood vessels formed during angiogenesis (Ahlskog et al. 2006; Carmeliet 2003; Folkman 1995). These radioimmunoconjugates exploit the exquisite targeting specificity of the humoral immune system to deliver lethal doses of cytotoxic radiation by the decaying radionuclides to the tumor. Although considered as one of the classic techniques, RIT has significantly progressed in the past decade due to several factors which include the large-scale availability of mAbs, humanization of mAbs,

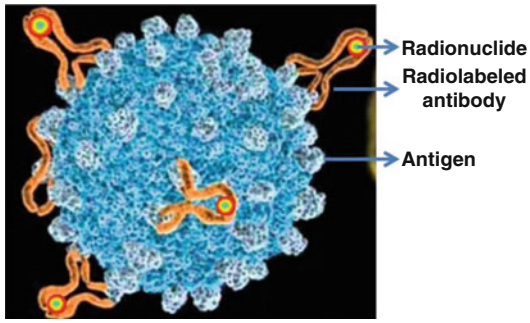

Fig. 9.5 Radiolabeled antibodies are targeted against various antigens which are overexpressed on the tumor cell surface

and development of new chelate molecules and novel-targeting techniques (Sharkey et al. 2003). This modality is primarily used for the treatment for hematopoietic malignancies which are very susceptible to radiation damage. Rapid targeting is effective for treatment of non-Hodgkin's lymphoma, which requires only low doses of radiation (~15 Gy) (Postema et al. 2001; Goldenberg 2001; Witzig 2001; White et al. 2000; Wiseman et al. 2001) (Fig. 9.5).

9.6.1 Advantages of RIT

The abundant and well-characterized cell surface antigens overexpressed on the cells in the case several major cancers offer the possibility of effective targeting in RIT. The ability to target specific antigens expressed on the surfaces of human cancer cells provides the prospect of using radiolabeled antibodies to guide the radionuclides to the tumor. In this sense, RIT provides a comprehensive route to the identification of tumor cells which generally overexpress antigens. Lymphomas are very sensitive to radiation in a dose-dependent fashion, and for this reason, they are ideal targets for RIT. The use of RIT offers the opportunity to attack not only the primary tumor cells but also lesions which are systemically metastasizing. The level of radiation that reaches the target—and thus the estimated radiation dose which will be delivered—can be accurately determined using pre-dose imaging techniques using low activity levels and therefore offers the possibility to administer higher radiation doses to the tumor site. In general, the levels of radiation to nontarget organs are predictable from imaging studies. The therapeutic effectiveness of RIT depends mainly on the radionuclide payload, rather than antibody-dependent cellular cytotoxicity and complement-dependent cytotoxicity (Kaminski et al. 1993; Trikha et al. 2002). Chapters 5, 6, and 7 describe the properties and production methods for these therapeutic radionuclides. Unlike unconjugated antibodies, radioimmunoconjugates can be effective when the host immune system is not fully functional, can destroy antigen-negative cells within tumors, and can overcome poor penetration of the antibody into tumors. The latter is possible with the use of appropriate radionuclide with required radiation penetration capability, as described in Chap. 2. This strategy thus offers the scope of treating patients who failed treatment with non-radiolabeled monoclonal antibodies. In this context, an important added advantage of radioimmunotherapy is the relevant "cross-fire effect." Since the radiation emitted from the radionuclides carried by the radioimmunoconjugate may be deposited in an area covering several cell diameters, poorly perfused or non-antigen-expressing cells within the tumor mass also could suffer the cytotoxic radiation effect (Fig. 9.6).

There are several instances when RIT is extremely valuable (Sautter-Bihl et al. 1996) and include residual micrometastatic lesions, residual tumor margins after surgical resection, tumors in the circulating blood including hematologic malignancy, and malignancies that present as free-floating cells.

9.6.2 Selection of Target Antigen

The choice of the target antigen plays a key role in determining the success of RIT. The antigen should be confined to the malignant cells for effective targeting and prevent a subpopulation of antigen-negative cells from proliferating, and for this reason, a favorable antigen expression profile is desirable for successful tumor targeting. To ensure specificity,

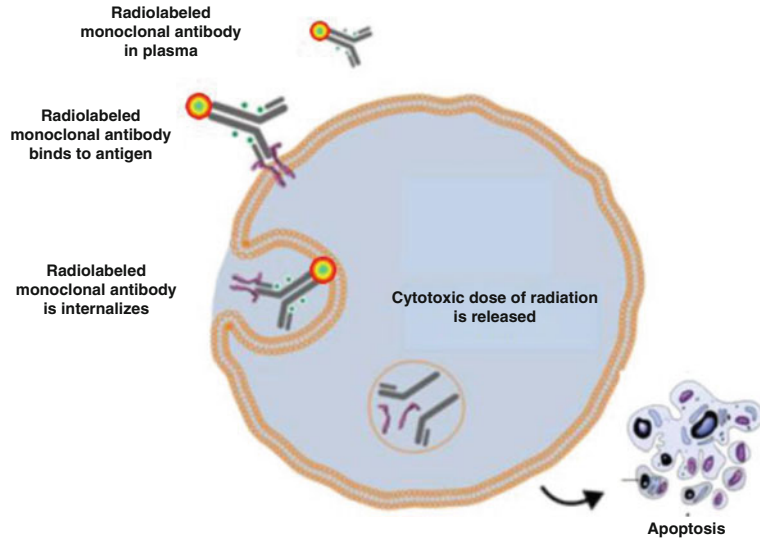

Fig. 9.6 Illustration of the primary mechanism of action of radiolabeled monoclonal antibodies for the targeted delivery of cytotoxic doses of radiation

the antigen must be overexpressed in target cells and have minimum presence in healthy cells. In terms of antigen location and binding stability, effective antibody-mediated cytotoxicity is achieved if the target antigen is not internalized or shed following antibody binding. In order to achieve effective therapy, a high density of antibody binding to the cell surface is essential, and of course antigens that shed from the cell surface and circulate in the peripheral blood at high concentration are not useful targets in these cases. Finally, the chosen antigen should not mutate in a way that would allow cancer cells to avoid destruction by the immune system. Based on the above criteria, a wide variety of antigens on cell surface have been considered as targets for a variety of tumors which fulfill such criteria to differing degrees (Caron et al. 1994; Goldenberg et al. 1981; Schlom et al. 1990) (Fig. 9.7).

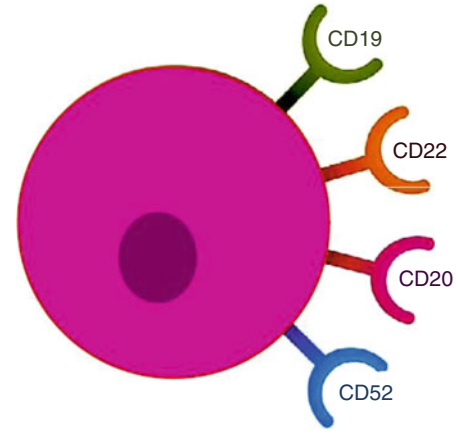

Fig. 9.7 Illustration of the expression of multiple cell surface epitopes

9.6.3 Antibody Selection

While RIT exists at the interphase of various disciplines, its success primarily depends on the selective accumulation of a cytotoxic radionuclide at the targeted site. The success of RIT therefore resides on the selection of the mAbs which must possess key features which include the ability to recognize target antigens with high affinity and specificity. The mAbs should also exhibit high binding affinity to the intended target, and binding should result in high tumor-to-background ratios. In order to be therapeutically useful, the radiolabeled antibody should have the ability to activate host effector functions such as complement-dependent cytotoxicity (CDC) and antibody-dependent cell cytotoxicity (ADCC). moAb binding of the radiolabeled antibody should induce apoptosis or inhibit survival signals in the targeted neoplastic cells. In

terms of the development of moAbs for therapy, monoclonal antibodies which are already approved for clinical immunotherapy applications are of course candidates for development of radioimmunotherapeutic conjugates, and this is indeed the strategy which has often progressed in the RIT field.

9.6.4 Selection of a Radionuclide for RIT

Selection of a radionuclide for RIT primarily focuses on the type of particle which is required, the energy of particulate emission, the physical half-life, and ability to be incorporated into the antibody either directly or through BFC agents. The choice of radionuclide (Chap. 2) is also influenced by the clinical disease, such as tumor size, physiological behavior, and tumor radiosensitivity. Radionuclides which decay with emission of beta radiation (β^-) are advantageous for RIT of solid tumors especially, owing to cost-effective availability and the "cross-fire" effect where surrounding cells not receiving sufficient antibody binding are also destroyed by radiation from adjacent targeted cells. A low abundance of low-energy gamma radiation emission is helpful for imaging and dosimetry. On the basis of in vitro cytotoxicity findings, in vivo studies, and upon theoretical dosimetry calculations, it is well established that α-emitting radionuclides (Chaps. 3 and 8) and Auger electron emitting radionuclides (Chap. 4) have the ability to treat even single tumor cells in the circulation, micrometastases, and, in certain cases, minimal residual disease (Humm 1987, 1996; Kozak et al. 1986; Zalutsky et al. 1996; Behr et al. 1998; Griffiths et al. 1999; Barendswaard et al. 2001). It has been suggested that shorter-range radionuclides are effective for the treatment of cancers, such as neoplastic meningitis and ovarian cancer (Goldsmith 2010). Characteristics of radionuclides with potential for use in radioimmunotherapy are discussed in detail in this chapter and in Chap. 10 and are summarized below in Table 9.4.

9.7 Treatment of Non-Hodgkin's B-Cell Lymphoma

As a key successful example of RIT in the clinical arena, as discussed earlier, lymphomas are malignancies of the lymphoid tissue which are broadly classified into Hodgkin's lymphoma (HD) and non-Hodgkin's lymphomas (NHL). Non-Hodgkin's lymphomas are a heterogenous group of lymphoreticular malignancies with a wide range of aggressiveness. The majority of NHL are B-cell lymphomas, with the follicular and diffused large B-cell lymphomas constituting up to 50 % of NHL (Azinovic et al. 2006). NHL provides an ideal candidate for RIT owing to its accessibility to the therapeutic antibodies as well as sensitivity to apoptosis, complement-dependent cytotoxicity (CDC), and antibody-dependent cellular cytotoxicity (ADCC)-mediated killing of cancer cells. The CD20 antigen is one of the many epitopes expressed on the mature B cells, making it a suitable target antigen for therapeutic radioactive monoclonal antibodies. CD20 is not present on stem cells and its expression does not vary at different stages of the cell cycle. Moreover, this antigen does not internalize or shed from the cell surface in response to antibody binding. By linking anti-CD20 monoclonal antibodies to an appropriate therapeutic radionuclide, disseminated disease sites can effectively be targeted (Schroff et al. 1985). Although several radionuclides have been considered for RIT, most attention has been focused on the use of iodine-131 (^{131}I) and yttrium-90 (^{90}Y). The relative advantages and disadvantages of these two radionuclides for RIT are listed in Table 9.5.

In 2002, ibritumomab tiuxetan (Zevalin®) radioimmunotherapy was approved by the US FDA for the treatment of patients with relapsed or refractory low-grade, follicular, or CD20+ transformed B-cell NHL and rituximab-refractory follicular NHL (Schroff et al. 1985). The antibody moiety of Zevalin®, ibritumomab, is a murine IgG1 kappa monoclonal antibody, targeting the same epitope on the CD20 antigen. Its chimeric counterpart is rituximab, a commercially available monoclonal antibody used for immunotherapy. Tiuxetan (1,4-methyl-benzyl

Table 9.4 Attractive radionuclides and their use in radioimmunotherapy

Key radionuclide examples	Half-life	Max range in soft tissue	Clinical use or animal model studies	Key features
β^--emitters				
^{90}Y	64.1 h	11.3 mm	^{90}Y-ibritumomab tiuxetan (Zevalin)	FDA approved for C20 positive non-Hodgkin's lymphoma
^{131}I	8.0 days	2.3 mm	^{131}I-tositumomab (Bexxar)	FDA approved for C20 positive non-Hodgkin's lymphoma
^{177}Lu	6.7 days	1.8 mm	^{177}Lu-LL1 antibody investigated in mice bearing B-cell lymphoma xenografts	Limited studies
α-emitters				
^{211}At	7.2 h	60 μm	^{211}At-Mov18 antibody investigated in mice-bearing human ovarian cancer	Limited availability
^{212}Bi	60.6 m	90 μm	^{212}Bi-B72.3 used in a murine model of human colon carcinoma	Short half-life may limit to locoregional applications
^{213}Bi	45.6 m	84 μm	^{213}Bi-HuM 195 in clinical trial for CD33 positive acute or chronic myeloid leukemia	Short half-life may limit to locoregional applications
Auger electron emitters				
^{125}I	60.2 days	<100 nm	^{125}I-A33 antibody used in phase I/II clinical trials in patients with advanced colon cancer	Long half-life may limit clinical utility
^{123}I	13.2 h	<100 nm	DNA-associated decay of ^{123}I shown to be effective at inducing DNA damage and cytotoxicity due to Auger component	Relatively high-energy γ-emission used for diagnostic imaging
^{111}In	8.0 days	<100 nm	^{111}In-anti HER2 antibodies shown to specifically induce cytotoxicity in human breast and ovarian cancer cell lines	Mainly used for imaging and dosimetry prior to therapeutic administration of Zevalin®

isothiocyanate diethylenetriamine pentaacetic acid, MX-DTPA), a modified chelator, is covalently bound to ibritumomab which allows chelating ^{111}In for imaging as well as ^{90}Y for therapy. In 2003, Bexxar®, a monoclonal antibody (tositumomab) labeled with ^{131}I, was approved by the US FDA for the treatment of CD20 positive, follicular NHL refractory to rituximab and has relapsed following chemotherapy. Tositumomab is a mouse monoclonal antibody and hence could result in HAMA response on repeated use. Iodine-131 is incorporated into antibodies by direct iodination of tyrosine residues. RIT with ^{131}I-tositumomab or ^{90}Y-ibritumomab tiuxetan has been reported to be an effective treatment option for patients with relapsed or refractory indolent B-cell NHL. Comparisons of their properties along with treatment details are elaborated in Table 9.6 (Michel et al. 2005; Goldenberg and Sharkey 2006). In a very insightful article, the diminished use of these agents in relation to the well-established patient benefit has even been reported in the main stream press (Berenson 2007).

Several other major cancers other than NHL are being targeted to be treated with radiolabeled monoclonal antibodies. Table 9.7 summarizes examples of many radiolabeled moAbs which have been used or RIT of cancer reported in this century (Kawashima 2014).

Table 9.5 Advantages and disadvantages of iodine-131 and yttrium-90 for RIT

Radionuclide	Advantages	Disadvantages
Iodine-131	Relatively inexpensive and readily available The gamma component makes it amenable to imaging using conventional gamma cameras	The long path length of the gamma component can result in increased exposure to hospital staff during treatment administration and follow-up Retention of radionuclide in the tumor is lower due to dehalogenation and release of the iodine from the targeted antibody ^{131}I that detaches from the antibody is typically taken up into the thyroid gland, and such accumulation could potentially lead to hypothyroidism as a late effect of treatment Large variability among patients in radionuclide excretion, thus requiring dosimetry for customized patient dosing
Yttrium-90	The beta emission from ^{90}Y has a longer path length than that of ^{131}I, which is advantageous in tumors with heterogeneous antibody distribution as it permits radiation to a larger area Since ^{90}Y has no gamma component, shielding of hospital personnel or using high patient doses are easily managed and do not require hospital stay after administration	Direct attachment to the antibody is not feasible; therefore, a bifunctional chelating agent is first attached to the antibody The lack of a gamma component requires bremsstrahlung imaging or the use of a surrogate radioisotope if an imaging component is desired. Typically, ^{111}In is used since binding to the targeting antibody conjugate is similar to that of ^{90}Y If freed from the chelated mAb, $^{90}Y^{+3}$ accumulates in bone, thereby increasing radiation to the marrow Requires on-site availability of a ^{90}Sr/^{90}Y generator. Relatively expensive and not widely available

9.8 Summary

Radioimmunotherapy (RIT) has emerged as one of the most promising therapeutic strategies for the treatment and management of hematologic malignancies and affords patients often with a high chance of achieving a potentially durable remission. Recent advances in radiochemistry, antibody technology, genetic engineering, and radiobiology provide important tools for RIT. An important RIT landmark was the FDA approval of radiolabeled anti-CD20 mAbs ^{90}Y-labeled ibritumomab tiuxetan (Zevalin®; Cell Therapeutics, Inc., Seattle, USA) and ^{131}I-labeled tositumomab (Bexxar®; GlaxoSmithKline LLC, Delaware, USA), although only Zevalin® is currently used in the USA for the treatment of relapsed or refractory low-grade B-cell non-Hodgkin's lymphoma and Bexxar® is evidently no longer available. In terms of use in the myeloablative setting, available data are encouraging, and phase III studies with Zevalin® are in progress. Although the exact role of such agents is still being established, newer radioimmunoconjugates are under development. Although dose-limiting toxicity of RIT is hematological, depending on bone marrow involvement and prior treatment, non-hematological toxicity is generally low. Reported studies are assessing innovative RIT protocols or new indications, in particular treatment in patients with aggressive lymphomas. High-dose treatment, RIT as consolidation after different therapeutic induction modalities, RIT in first-line treatment, or fractionated RIT showed promising results. RIT is coming of age at a time when molecular medicine is becoming a reality. New mAbs, in particular humanized mAbs, or combinations of naked and radiolabeled mAbs, by themselves, are finding their place in cancer therapy, as are small molecules that modulate a variety of processes, from cell surface receptor expression to intracellular enzyme activity. Personalized dosimetry protocols should be developed to determine injected activity.

Table 9.6 Comparison of Zevalin® and Bexxar®

Properties	Zevalin®	Bexxar®
Radioisotope	^{90}Y	^{131}I
Type of radiation	β^-	β^- and γ
Max beta energy (mean)	2.29 MeV (0.9 MeV)	0.6 MeV (0.19 MeV)
Path length in soft tissue	0.8 mm	5.3 mm
Isotope half-life	64 h	8 days
Source of radionuclide	$^{90}Sr/^{90}Y$ generator	Reactor production
Availability of radionuclide	Limited	Widely
Antibody	Murine IgG-1 kappa antibody to CD^{20+}	Murine IgG2a lambda antibody to CD^{20+}
Ease of labeling	More complex	Ease
Pre-infusion antibody	Chimeric (rituximab)	Murine (tositumomab)
Pre-infusion dose	250 mg of rituximab	450 mg of tositumomab
Tracer imaging	5 mCi of ^{111}In	5 mCi of ^{131}I
Clearance	Urinary	Urinary, more rapidly
Purpose of diagnostic scan using tracer	1–2 scans to visually assess distribution	3 scans to determine clearance and determine therapeutic dose
Therapeutic dose	0.4 mCi/kg (maximum of 32 mCi)	Dose to deliver 75 cGy to total body dose
Critical organ	Spleen, testes	Thyroid
Dose determination	Fixed based on weight and platelets (0.4 or 0.3 mCi/kg)	Clearance rate/dosimetry based to deliver 75 or 65 cGy total body dose
Pre-therapy preparation	Antihistamines/NSAID	Additional thyroid blocking
Hematological toxicity	Predominant toxicity	Predominant toxicity, less severe than Zevalin
Other unique toxicities	Dehalogenation in liver and effect on marrow	Hypothyroidism
Radiation precautions	Universal for 1 week	Additional precautions for gamma radiation
Therapy setting	Outpatient	Majority outpatient

Table 9.7 Examples of radiolabeled anticancer agents for RIT

Cancer	Target molecule	mAb	Radioisotope	Subject
Direct radiolabeling methods[a]				
Non-Hodgkin's lymphoma	CD20	Ibritumomab	Y-90	Human (in clinical use)
		Tositumomab	I-131	Human (in clinical use)
		Rituximab	I-131	Human (phase II)
	CD22	Epratuzumab	Y-90	Human (phase II)
Myeloid leukemia	CD33	Lintuzumab	Bi-213	Human (phase II)
Raji B lymphoma	CD74	L243	Ga-67	Cell
Colorectal cancer	Carcinoembryonic antigen (CEA)	cT84.66	Y-90	Human (phase I)
	A33 glycoprotein	huA33	At-211	Mouse xenograft model
Colorectal cancer (liver metastases)	CEA	F6 $F(ab')_2$	I-131	Human (phase II)
	CEA-related cell adhesion molecule	Labetuzumab	I-131	Human (phase II)
Gastrointestinal cancer	CEA	A5B7	I-131	Human (phase I)

(continued)

Table 9.7 (continued)

Cancer	Target molecule	mAb	Radioisotope	Subject
Breast cancer	HER-2	Trastuzumab	Y-90	Mouse xenograft model
			Pb-212	Human (phase I)
		NLS-trastuzumab	In-111	Cell
Ovarian cancer	Na-dependent phosphate transporter	MX35 $F(ab')_2$	At-211	Human (phase I)
Prostate cancer	MUC-1	m170	Y-90	Human (phase I)
		J591	Y-90	Human (phase I)
			Lu-177	Human (phase I)
			Bi-213	Mouse xenograft model
Multiple myeloma	CD138	Anti CD138 Ab	Bi-213	Mouse xenograft model
Indirect radiolabeling methods[a]				
Non-Hodgkin's lymphoma	CD20	TF4 (HSG)	Y-90	Mouse xenograft model
		1 $F5(scFv)_4$ (streptavidin)	Y-90	Mouse xenograft model
	CD20, CD22, HLA-DR	Corresponding Abs (streptavidin)	Y-90	Mouse xenograft model
Colon cancer	CEA	hBS14 (HSG)	Y-90	Mouse xenograft model
		MN14 (MORF)	Re-188	Mouse xenograft model
	Ep-CAM	NR-LU-10 (HSG)	Y-90	Human (phase II)
Gastrointestinal cancer	TAG-72	CC49-$(scFv)_4$ (streptavidin)	Y-90	Human (phase I)
Glioma	Tenascin	BC4 (biotin)	Y-90	Human (phase II)

[a]Direct radiolabeling refers to methods used to attach the radioisotope directly to the antibody macromolecule. For indirect radiolabeling, the radioisotope is attached to another chemical moiety which is attached to the antibody

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