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MONOCLONAL ANTIBODIES AND ITS APPLICATIONS : AN OVERVIEW

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Almost eight decades ago Paul Ehrlich proposed the potential use of antibodies as carriers of bioactive agents to the target sites, and was known as "magic bullet" concept. Although considered promising, early attempts to apply this concept were largerly unsuccessful due to inability to prepare large quantities of homogenous antibodies with defined specificity. But now-a-day, with the development of hybridoma technology, it is possible to produce virtually unlimited quantities of homogenous antibodies having a defined specificity i.e. monoclonal antibodies, which have found use in sensitive immunodiagnostic tests[1-3]. The introduction of hybridoma technology by Kohler and Milstein in 1976 has transformed the whole field of immunology within a brief five-year period. There is hardly an immunology laboratory that is not using monoclonal antibodies as reagents in one form or the other. Though it is true that the concept of targeted therapeutic system is gaining more and more interest in the pharmaceutical as well as in medical community, however, the therapeutic use of monoclonal antibodies (MoAbs) and their conjugates is still in its infancy. This arcticle gives an insight primarily into the concept of the monoclonal antibodies and its application in therapeutics and drug targeting.

Keywords: *Monoclonal antibodies; Hybridoma technology; Drug targeting; Radioimmunoassay; Tumor*

Basic concept

An antibody is an immunoglobulin synthesised by the body's immune system in response to a foreign molecule (antigen) and capable of binding the antigen with specificity. Therefore, an antigen is an antibody generator and a molecule which is capable of forming antibody via host immune system. Generally, an antigen must have a relatively large molecular weight (>1000) in order to elicit an immune response and a smaller molecular can be made to be antigenic by coupling with a suitable macromolecule such as albumin.

An antibody is a Y-shaped molecule (Fig.1)[4] which contains two light chains and two heavy chains joined by disulfide bonds. Carbohydrate residue content is also present in each of the heavy chains. The bottom 'trunk' portion of the antibody molecule is known as the constant (Fc) region due to its amino acid sequence being often similar within a given animal species. The upper 'arms', the antigen-binding regions, (Fab), are known as variable regions since the amino acids sequence is determined by its antigen responsible for its formation. The variable region has several 'hypervariable' regions, which are also known as the complementarity determininig regions (CDR), which show greater variability than the rest of the variable region. Based on the literature informations antibodies are classified as follows:

1-*Polyclonal antibodies*: After an antigen is injected into an animal by a regimen designed to induce an optimal immune response, serum can be collected and the immunoglobulin fraction isolated. These 'antisera' are enriched with antibodies specific for the original antigen. Since large numbers of lymphocytes are involved in the production of antisera, antibodies produced by this classical method are called polyclonal.

2-*Monoclonal antibodies* : An antibody is called 'monoclonal' when each immunoglobulin is produced by a single clone of cells and hence is identical to every other molecule in the preparation in terms of heavy as well as light chain structure. MoAbs offer more consistent

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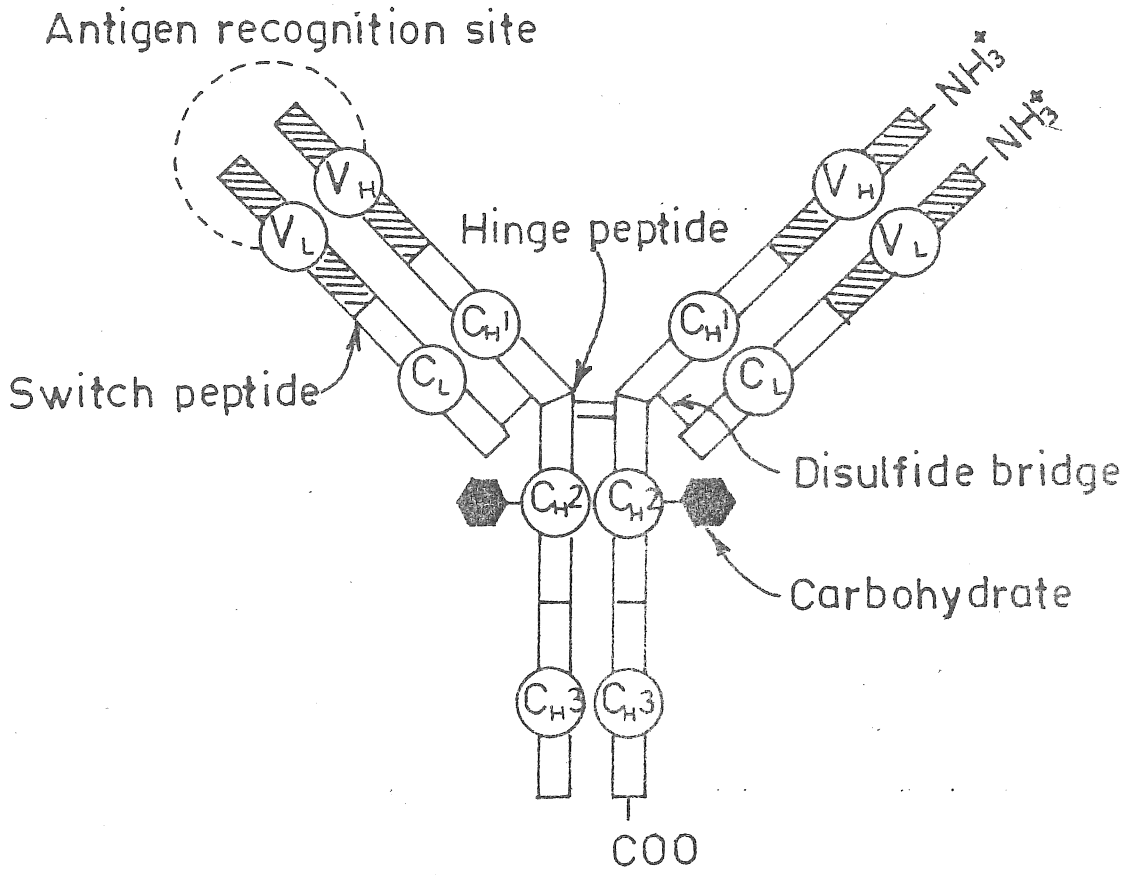

Fig.1.Monoclonal antibody molecule

efficacy and predictable toxicity *in vivo* than the polyclonal counterpart[5].

To understand the usefulness of MoAbs, it is necessary to understand the drawbacks of conventional polyclonal antisera-antibodies. The preparation of polyclonal antibodies, highly specific antisera, is unreliable and difficult. It also requires highly purified antigens and different lots of antisera have different specificities and affinities towards the antigen. On the other hand, MoAbs are highly specific and unlimited quantities of such antibodies can be produced against virtually any molecule, regardless of the purity of immunising antigens.

3-*Antibody fragments* : The earliest MoAbs examined in animal and clinical studies were murine antibodies. Because of their nonhuman origin, they are immunogenic in humans; that is, they have a tendency to elicit a human antimouse antibody (HAMA) response. Murine antibodies have been shown to have much shorter clearance rates than human MoAb. One approach to overcome this problem has been to cleave the antibody into its respective Fc and Fab fragments (Fig.2a)[6]. In general, the Fab fragments are less immunogenic than the corresponding intact antibodies, and their smaller molecular size may facilitate penetration into tumor tissue[7] and result in a longer half-life. They can lose some of their antigen-binding capacity and in some cases the therapeutic effect may depend on the Fc portion of the antibody.

4-*Chimeric antibodies*: The obvious solution to the problems encountered with

murine antibodies would be to clone a fully human antibody. However, human hybridomas required for the human MoAb production have been notoriously difficult to culture and it may be impossible to obtain many of the appropriate antibodies. A strategy has been devised to overcome the HAMA problem of murine MoAbs by constructing a chimeric antibody (Fig.2b) [6], which contains the Fc region of human IgG, but the Fab regions are murine in origin. These can be made chemically by joining murine Fab fragments to the human Fc fragment, but the preferred technique is to use recombinant DNA technology.

5- *Humanised antibodies*: Although chimeric antibodies appear to elicit less HAMA response than murine antibodies, they are still immunogenic because around 30% of the total molecule is occupied by the murine regions. A major breakthrough was achieved when it was recognised that only a small portion of an antibody molecule was actually responsible for antigen binding, in fact only the CDR regions. One can envision construction of a 'humanised' antibody in which most of the antibody framework is human in origin, but the CDR's are murine (Fig. 2c)[6]. These humanized antibodies can be synthesized by recombinant DNA technology.

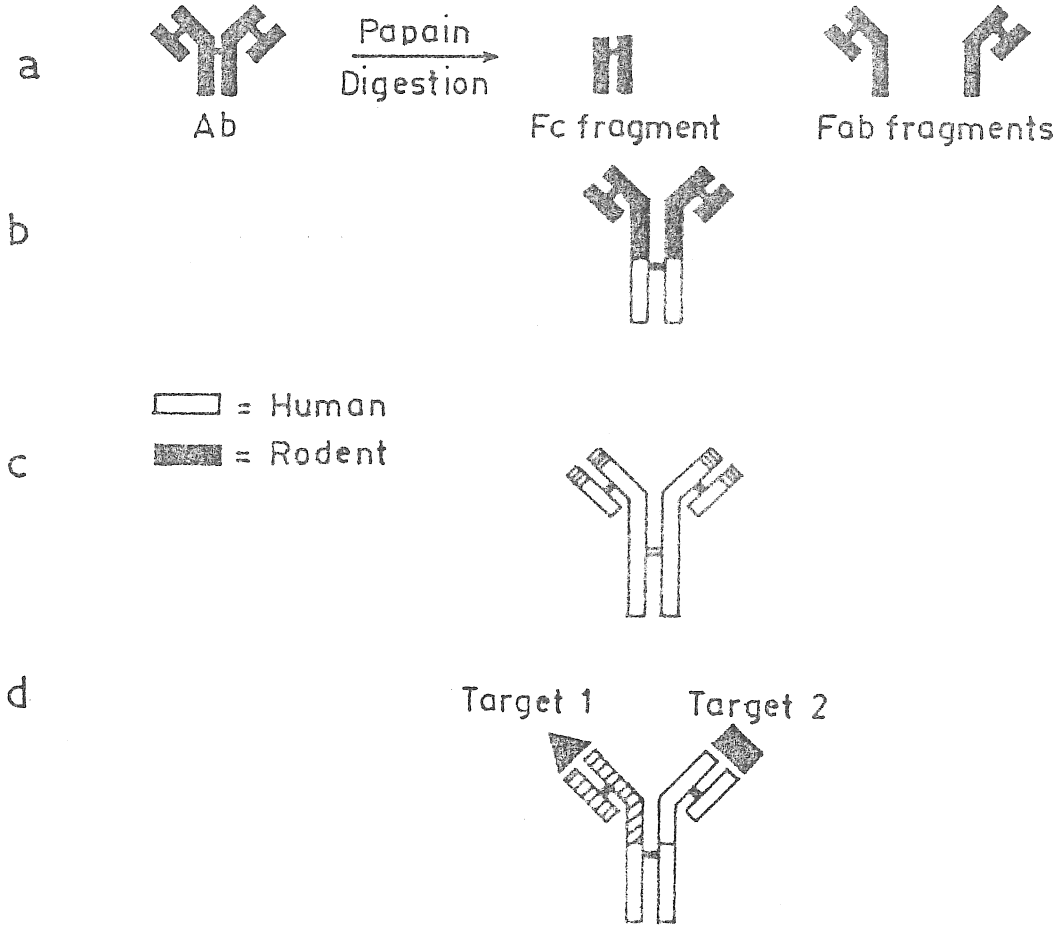

Fig.2. The most important monoclonal antibody constructs used clinically: (a) antibody fragments (b) chimeric antibody (c) humanized antibody (d) bispecific antibody

6- *Bispecific antibodies*: Antibodies can be constructed by recombinant DNA technology in which each of the two arms is specific for two different antigens (Fig. 2d). For instance, bispecific MoAbs reactive with CD 15 antigen and composed of Fab fragments of anti-CD64 MoAb 32 and a whole IgM antimyeloid cell MoAb, PM-81, have been investigated for the therapy of CD 15-positive tumors[8].

7-*Immunoconjugate*: For MoAb-targeted drug delivery, a drug is bound covalently to an antibody which is selected to target it to the desired site of action. The resulting immunoconjugate may contain a spacer between the drug and the antibody, or a polymer to increase the number of drug molecules that can be bound to each antibody. Another possibility is a radioimmunoconjugate, which is designed to be concentrated at the target site by the targeting antibody, allowing the radiation to exert its cytotoxic affect. Another possible alternative includes noncovalent incorporation of drug into a liposome or microsphere to which the targeting antibody is bound via the surface, yielding an immune-liposome or immunomicrosphere.

Applications

Applications in immunological research:

In both basic and applied immunological research MoAbs have wide application. For the former, the cluster of differentiation (CD) system plays a major role which is entirely defined by MoAbs and now encompasses more than 80 lymphocyte markers, to appreciate the immense value of MoAbs in identifying cells of the immune system together with their activation and differentiation markers. In combination with flow cytometric analysis (FACS), these CD specific MoAbs can be used to detect the appearance or absence of cell populations during antigenic stimulus or infection, for example in monitoring the decline of $CD4^+$ cells during the progression of AIDS infection, or the increase in cell surface density of lymphokine receptors during lymphocyte activation. In many ways, this may be considered to be their most important application to date.

Also, MoAbs are considered to have potential in clinical immunological research in the immortalization of B cell clones which have been inappropriately activated in disease such as autoimmune disease to study, for example, variable gene usage. It is, however, possible that in such cases the fault may lie within the T lymphocyte compartment with inappropriate help being given, and in this context MoAbs to the T cell receptor Vβ chains have been of considerable value in the analysis of the relationships between MHC antigens, antigen presentation and the presence or absence of the appropriate T cell receptors.

Diagnostic applications in general

In many fields MoAbs are now extensively used in diagnosis and the estimated annual market is in excess of 3000 million. In most cases, the double sandwich MoAb technique where a MoAb directed to one part of the antigen under test is used to capture it and a MoAb to a different epitope, linked to a detecting system, is used to provide the signal, is superseding radioimmunoassay. Diagnostic kits utilizing MoAbs for the detection of human chorionic gonadotrophin (hCG) in pregnancy or luteinizing hormone (LH) during ovulation are available on the open market and other home diagnostic kits for bacterial infections including streptococcal infections of the throat and venereal disease have been developed although these are not for open sale. The majority of current MoAb based kits utilize a color reaction as the final detection system, but these

can readily be adapted by luminescent are bioelectronic mechanisms to biosensors which can bleep, flash, or trigger mechanisms which generate further intervention without direct human involvement in the relevant decisions. Such systems may prove of value in continuous monitoring of environmental hazards or in process control during large-scale production of antibiotics. In the standard laboratory environment, MoAbs are now routinely used to determine ABO blood group antigens and blood and tissue levels of protein and polypeptide hormones. They are also extensively used to type and subtype bacterial, viral, and parasitic infections.

Applications in tumor diagnosis and therapy

In early 1980s, it was anticipated that the potentially high specificity of MoAbs would mean that they could be employed not only to identify small metastatic populations of tumor cells but also to kill them, either alone or in conjunction with a cytopathic agent, while leaving normal cells unharmed. This anticipation has largely not yet been realized and indeed many of the claims of tumor associated epitopes defined by MoAbs in the early 1980s remain unsubstantiated. None of the less, MoAbs have led to a much greater understanding of the nature of malignancy. Many of the problems have stemmed from the difficulties described above in generating human MoAbs and the consequent to employ rodent or human-rodent chimeric ones in therapy. Reports of successes with either have been sporadic and the former leads to side-effects and limited utility due to the rejection of the rodent antibody by the human immune system.

Other Applications

These tend to encompass improvements in technology achieved by transfer of experimental systems from polyclonal to monoclonal application and include catalytic monoclonal antibodies that performs reactions for which there is no natural enzyme system, and the use of monoclonal antibodies in antigen purification.

Conclusions

The field of MoAb-based drug delivery is much more complex than probably evidenced from the above discussion. One potential problem is that the MoAb will not be specific *in vivo* as would be predicted from *in vitro* studies. In some cases, pack drug concentration with MoAbs have been found to be only two to three times higher in tumor tissue than the surrounding normal tissues[9]. Several studies have demonstrated localisation of antitumor MoAbs in tumor tissue at levels more than five-fold higher than in normal tissue, but equally frequently with MoAbs demonstrating high affinity for tumor cells *in vitro* have been shown to lack specific binding with tumor cells *in vivo* [10-13]. Current literature suggests that the availability of high affinity MoAbs that recognise specific antigens without cross-interaction with normal cells is still scarce. The only exception to this observation is the surface-immunoglubulin idiotype expressed by certain B-cell lymphomas[14].

The presence of circulating tumor-associated antigen is one of the factors which may reduce the overall efficacy of MoAb-directed delivery systems and complicate their evaluation. For instance, the presence of circulating carcinoembryonic antigen has been shown to complicate the application of MoAbs against this antigen[15]. In view of these problems, MoAb-directed delivery system may ultimately be restricted to those few cases in which there are relatively

high densities of known antigens in all cells of the target site. Another problem in targeting is the heterogeneity of tumor cells, that is a specific antigen may not be present in sufficient quantities in all cells of the target tissue to allow selection of suitable antibody. Recently, a method was proposed for MoAb-based drug delivery to target areas with heterogeneous antigens[16]. The proposed method requires sequential administration of a mixture of modified antibodies against different antigens in the target area followed by administration of a drug carrier which recognises, and interacts with accumulated antibodies[16] (Fig.3). The practical feasibility of this strategy was confirmed following administration of a mixture of biotinylated antibodies to target components followed by administration of biotinylated and avidin-bearing liposomes. The binding of biotinylated liposomes via avidin was found to be higher than that achieved with liposomes bearing a single antibody[16].

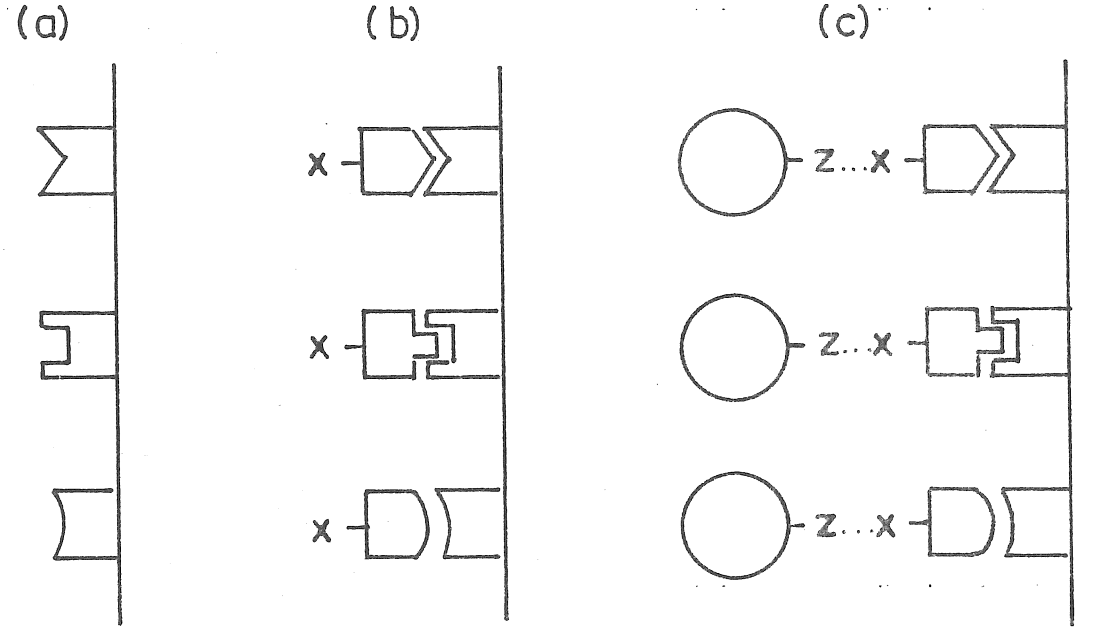

Fig.3. A schematic representation of the unification of delivery systems to optimize therapeutic outcome with MoAbs; (a) exposed target antigens, (b) initial treatment with bridge molecules, and (c) specific binding of unified carrier systems

The uptake of MoAb-based delivery systems by the reticuloendothelial system (RES) is another drawback of these systems. It has been suggested that only 0.1 to 1% of the administered dose of antibody-based systems reaches non-RES sites, with less than 8% dose reaching non-RES sites under optimal situations[17]. Apart from this, the immunogenicity of 'foreign' MoAbs has always been a major factor in the lack of success of MoAb-based therapeutic systems. It has been suggested that the cleavage of the Fc fraction of MoAb and the use of Fab_2 fragment may improve drug delivery without sacrificing the specificity of antigen-MoAb binding because elimination of Fc portion would likely reduce immunogenicity and nonspecific binding to normal cells [18]. Indeed Fab_2 fragment alone has been shown to be less toxic than intact IgG in mice bearing a

tumor against which the antibodies were directed [19]. Furthermore, immunotoxins based on Fab_2 have been shown to be more effective than the ones based on Fab. Improved endocytosis of Fab_2 versus Fab, along with its superior binding affinity with the target cells antigens, have been suggested to be the possible reasons for this observation [20].

Humanised antibodies hold the greatest promise in reducing the immune response of MoAb-based therapies. Although immunotherapy with these entities is promising and indicate greatly decreased immune responses. Immunoliposomes and similar carrier systems overcome some of the problems by maximizing the payload-antibody ratio. Since the number of antigen-binding sites on the target tissue is often limited, it has been suggested that immobilisation of several antibodies against the target-cell surface determinants may provide cooperative multiple-point binding of immunocarries to target cell [21].

All the above-discussed complexities seriously limit the routine application of antibodies in drug delivery. Under these circumstances, it is important to develop systems that allow transvascular delivery of toxic compounds without regard of tissue permeability. An ideal system should allow efficient encapsulation of the intended compound in order to protect its degradation prior to and during endothelial transfer and hence minimise inherent toxicity and allow its controlled release in the extravascular compartment of target tissue.

New applications in the field of antibody directed drug delivery may be developed by combining the technology with another form of targeting or other means of optimisation. Another recent approach combines MoAb targeting with enzymatic prodrug activation. In this therapeutic method, called antibody-directed prodrug therapy (ADPT), an enzyme-antibody conjugate is administered and allowed to accumulate in the target site (e.g. tumor).

Despite the problems discussed in this article, MoAbs should hold an important place in drug delivery and therapy in future. Although the number of therapeutic applications that will eventually be suitable for this technology may be small, the problems should not be insurmountable and these applications may yield important advantages over other therapies. Proper attention to detail must be paid in the choice of antibody, coupling method, drug, route of administration, dose and factors in order to design an effective therapy for a particular disease. The mechanisms of distribution, uptake metabolism and pharmacological effect must be properly understood to develop a rationale for particular MoAb directed therapy. Nevertheless, it is likely that next decade will see a number of MoAb-directed therapies reaching extended clinical trials and perhaps come to the pharmaceutical market.

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