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Chapter 32. Humanized Monoclonal Antibodies

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Introduction - Almost two decades ago, the development of monoclonal hybridoma technology — the ability to make cultured cells produce antibodies of predefined specificity — promised a new weapon in the arsenal of molecules able to combat disease (1). Antibodies against a specific antigen (or target molecule) could be generated, incorporated into a hybridoma for production, and then used in diagnosis or therapy. However, only rodent monoclonal antibodies could be made due to technological limitations. In most clinical applications, these rodent monoclonal antibodies exhibit properties which severly limit their utility. First, they may induce an immunogenic response in humans, referred to as HAMA (human anti-mouse antibodies), when the human immune system recognizes them as foreign substances. Second, the therapeutic efficacy of the rodent antibody may also be reduced because a rodent antibody is cleared from serum more rapidly than human ones. Third, in humans, rodent antibodies generally exhibit only weak recruitment of effector functions, such as antibody-dependent cell-mediated cytotoxicity and complement fixation, which may be requisite for the function of the antibody. Use of a human antibody would circumvent these three problems.

With advances in molecular biology and mammalian tissue culture, it became possible to obtain useful amounts of any antibody, thereby surmounting the limitations of hybridoma technology and use of only rodent antibodies. But one problem still existed — how does one obtain *human* antibodies against a particular human antigen? Even if one could ethically use a human subject as the biological factory, the human immune system, in general, does not produce antibodies against human proteins. Two techiques have been developed to address this problem. Earliest of these was the construction of chimeric antibodies in which entire antigen-binding domains from a rodent antibody are substituted for those of a human antibody (Fig. 1) (2). In many cases, these molecules still exhibited some, albeit reduced, HAMA. The next step was generation of humanized antibodies in which a significantly reduced number of rodent residues are incorporated into a human antibody such that the humanized antibody has the same binding characteristics and specificity as the original rodent antibody (3-5). In order to understand the technique of humanization and the difference between a chimeric and humanized antibody, the structure of antibodies must be appreciated.

Antibody Structure - An antibody consists of four peptide chains — two identical light chains and two identical heavy chains — which form a 'Y' shape. The functions of the antibody reside in different domains. Antigen binding occurs at the ends of the arms of the 'Y', each arm being referred to as a Fab or antigen binding fragment. Hence each antibody can bind two antigen molecules. The effector functions reside in the base of the 'Y', referred to as the Fc portion. Each light chain consists of one variable domain and one constant domain; each heavy chain consists of one variable domain and three constant domains (Fig. 1). The 'constant' notation refers to the fact that for a particular species and immunoglobulin class (e.g. IgG, IgE, IgA) the amino acid sequence for the constant domain

is, for our purposes, invariant. The 'variable' notation refers to the fact that certain portions of variable domains differ extensively in sequence among antibodies and are used in the binding and specificity of each particular antibody for its particular antigen. These portions are referred to as complementarity-determining regions (CDRs). Both light and heavy chain variable domains contain three CDRs to give a total of six CDRs involved in binding antigen. Each CDR is a contiguous sequence of amino acids which form a loop connecting two β-strands of the framework. Note though that the amino acid sequence of the non-CDR portion of the 'variable' domain is relatively invariant and is used to categorize these domains into subgroups (6).

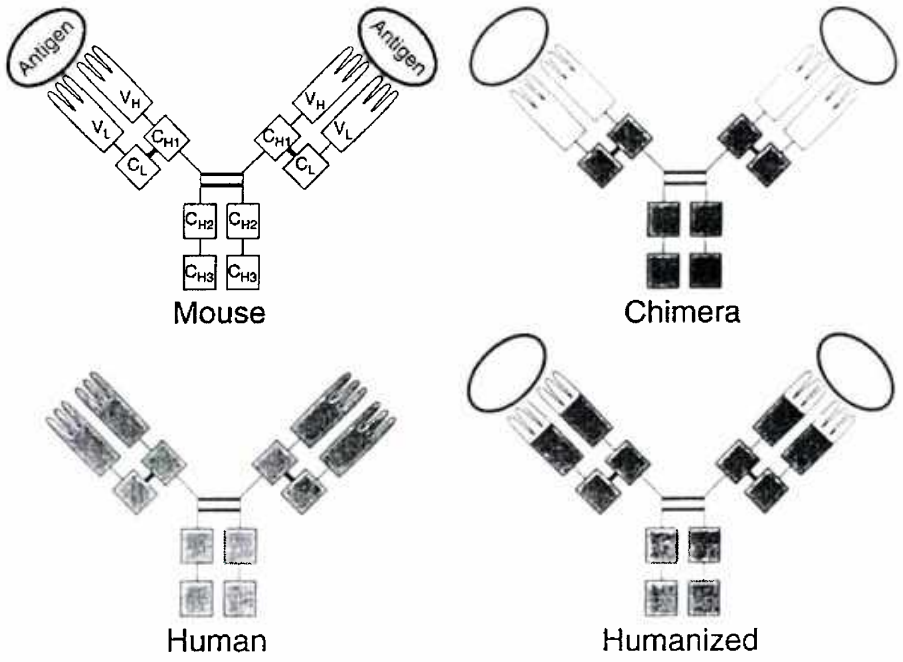

Figure 1. Chimeric and Humanized Antibodies. CDRs are represented by 'fingers' on variable domains of the light chain (V_L) and heavy chain (V_H). Note that only the mouse, chimera and humanized antibodies bind antigen. Disulfide bonds between the two heavy chains and between the light and heavy chains are denoted by thicker, dark lines.

In one type of chimeric antibody all of the variable domains from a rodent antibody are fused onto the constant domains of a human antibody (Fig. 1). This type of chimeric antibody is comprised of four rodent domains and eight human domains and consequently retains approximately 33% rodent residues. In the corresponding humanized antibody, only the six rodent CDRs replace the six human CDRs (Fig. 1). The resulting antibody contains only about 5-10% rodent residues and is still chimeric in that it has residues derived from different species. From the perspective of the human immune system, the humanized antibody is the more 'human' of the two types as it contains fewer rodent residues. Moreover one must appreciate that the amino acids in CDRs are highly variable and this variance is independent of species. The nature of the CDRs is dependent upon the target, whereas the framework is species dependent. Hence even though the humanized antibody still retains about 5-10% rodent residues, these residues would also be variant in human as

well as rodent and thus the humanized antibody could well be very similar to a human antibody directed against the same site on the same target as the original rodent antibody.

Humanized Antibody Construction - At first glance constructing a humanized antibody is rather simple and straightforward. One chooses a human antibody, clips off the six human CDRs and replaces them with their structurally analogous rodent CDRs. Indeed some humanized antibodies have approached this simplicity in method (7,8). However, there are both theoretical and technical difficulties. Among the former is the exact definition of a CDR, i.e. exactly which residues comprise the CDRs and which comprise the framework. Two definitions have been proposed. The first relies solely on analyses of the amino acid sequences of a multitude of antibodies (6). The variance of amino acid types at each residue position of antibodies was evaluated and those positions which exhibited a relatively large variance were included in a CDR as long as they were contiguous to other positions with high variance. Residue positions showing relatively low variance were termed framework. The second definition was derived from structures of antibodies (9). When the two definitions are compared some striking differences are apparent. While the sequence-based CDRs are generally larger than, and encompass, the structure-based CDRs, a notable discrepancy occurs in the definition of CDR-H1 (i.e. the first CDR loop in the heavy chain sequence) where the two definitions overlap by only two residues. Generally, the sequence-based definition of CDRs has been used when an antibody is humanized.

The technical difficulties fall into three categories. First, the choice of constant domains must be made. To date almost all antibodies humanized have been those of the IgG class. In humans there are four recognized subclasses, denoted IgG1, IgG2, IgG3 and IgG4, the classification of which is based on the sequence of their constant domains. Each subclass also exhibits differences in effector, or biological, functions mediated by the constant domains. The intended therapeutic use of the antibody must be considered when choosing the IgG subclass, e.g. is complement fixation an advantage or disadvantage for the particular application?

Second, the choice of human variable domains, both light and heavy, must be made. In the homology or 'best-fit' method, the sequence of the rodent variable domain is screened against the entire library of known human variable domain sequences. The human sequence which is closest to that of the rodent is then accepted as the human framework (8,10). Another method uses a particular framework derived from the *consensus* sequence of all human antibodies of a particular subgroup of light or heavy chains. The same framework may be used for several different humanized antibodies (11,12). Remembering that the purpose of humanization is to trick the human immune system into recognizing the humanized antibody as one of its own, is the 'best-fit' or the consensus framework better for this trickery? Unfortunately there are very limited *in vivo* data on the reaction of the human immune system to humanized antibodies and no comparative study has been performed on the two types. In the final analysis both may function well with regard to acceptance by the human immune system, with perhaps an occasional aberration.

Third is the most imposing technical difficulty -— designing the humanized antibody such that it has the same binding affinity and specificity as the original rodent antibody. When the rodent CDRs are simply grafted onto a human framework the resultant humanized antibody may exhibit signficiantly reduced binding compared to the original rodent antibody (11,12). This reduction in binding may be caused by certain buried framework residues. Each CDR is a loop and these loops may be anchored to the framework not only covalently at their ends but through noncovalent interactions with the sidechains of buried framework residues. For example, in CDR-L1 the sidechain at position 29 (residue numbering is according to ref. 6) is usually hydrophobic, buried and contacts hydrophobic sidechains at framework positions 2, 25, 33, 71 and 90 (13). If an incorrect amino acid is chosen for any of these framework positions the packing of CDR-L1 against the protein might be altered, resulting in an incorrect presentation of the exposed CDR-L1

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