FEBS Letters 430 (1998) 92–94 FEBS 20392

Minireview

Synthetic human antibodies and a strategy for protein engineering

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Received 18 May 1998

Abstract Our understanding of the way antibodies are built in vivo has provided an approach for engineering synthetic human antibodies in bacteria. Such antibodies have not only been raised against foreign antigens, but also against highly conserved antigens or human self-antigens, and have considerable practical potential as reagents for research and also as therapeutics. The approach also has implications for the design of antibody repertoires and for engineering other proteins with desirable binding properties. This review takes a personal view.

Key words: Antibody engineering; Synthetic antibody; Phage display

1. Introduction

The discipline of the organic chemist is that the determination of the structure of a natural molecule is not regarded as complete until the same molecule has been chemically synthesised. It is usually difficult to apply such disciplines in the biological sciences. However our understanding of the way antibodies are made in vivo has inspired the creation of an artificial immune system that has proved capable of making human antibodies in bacteria [1,2] (Fig. 1).

The antigen binding site is comprised of two associated domains, the heavy (H) and light (L) chain variable domains, which together form a β-sheet framework for six antigen binding loops. The strategy of the immune system for making antibodies is to create a large repertoire of antibody structures (differing in the sequences and structures of the loops), and to select out those with the required antigen binding activities.

The repertoire of antibodies is created during B-cell development by DNA rearrangement and combinatorial assembly of different genetic segments [3]. The heavy chain variable genes are assembled from the combination of members of three different classes of segments (VH, D and JH); the light chain variable genes from two classes of segments (Vλ and Jλ; or Vκ and Jκ). Each of the V segments contributes two of the hypervariable loops. The third loop is contributed by the junctions of the segments and further sequence diversity is provided by the D segment (in the case of the heavy chain) and trimming and addition of nucleotides at the junctions. Recently the human VH, Vκ, Vλ and D segments have been mapped, cloned and sequenced. We now know that human antibody heavy chains can be assembled from any of about 51 VH, 25 D, and 6 JH segments, and human light chains from any of about 31 Vλ and 4 Jλ (or 40 Vκ and 5 Jκ) segments (for a compilation, VBASE, see www.mrc-cpe.cam.ac.uk/imt-doc/public/INTRO.html).

The antibodies encoded by the assembled segments are expressed on the surface of B-lymphocytes. Each lymphocyte bears a single antibody but the population of lymphocytes (about 10^7 lymphocyte clones in a mouse or more than 10^9 in a human) constitutes a large repertoire of different antibody structures. On binding to antigen a selected lymphocyte is triggered into differentiation leading to a plasma cell that secretes soluble complementary antibody into the serum. The binding affinities of antibodies selected from such a primary repertoire are modest (μM for the mouse). However the antigen-selected lymphocytes also differentiate into memory cells (that continue to display surface antibody), and in this process the rearranged V genes are subjected to random somatic mutation. On further challenge with the same antigen, memory cells bearing mutant antibodies with improved binding affinities are selected (by competition for limiting antigen). In this way the binding affinities can be improved by several orders of magnitude [4,5].

2. Creating the repertoire

To mimic the strategy of the immune system, we first need to create a repertoire of antibody genes. Repertoires can be created by cloning the natural rearranged human V genes from peripheral blood lymphocytes [6,7]; however this article will focus on the creation of synthetic repertoires directly from (unrearranged) V gene segments. Thus an entirely synthetic repertoire of human variable region genes was created by assembly of each of the human VH segments with D segments of random nucleotides together with a human J segment [8]; likewise each of the human Vλ (or Vκ) segments with a Jλ (or Jκ) segment [9].

Filamentous bacteriophage provides a means of display and selection of peptides [10,11] and proteins [12,13], and in particular antibody fragments with binding activities. Thus in the genes encoding the antibody heavy and light chain variable domains are linked (as 'single chain Fv fragments') [14,15] and fused to the gene encoding the minor coat protein p3, the phage encapsidate the antibody genes and display the 'single chain Fv fragment' on the surface [13]. The genes encoding antibody fragments of predefined binding specificity can therefore be isolated by binding of the phage to antigen-coated surfaces. By using several cycles of selection (and growth of the enriched phage in bacteria between cycles), it is possible to isolate a single binder from $>10^9$ non-binders. Antibody fragments can also be assembled as two chain Fab fragments, by fusion of one chain to the phage and secretion of the other chain into the bacterial periplasm [16,17]. The filamentous phage, like the B-cell, provide a vehicle for linking the anti-

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PII: S0014-5793(98)00628-0

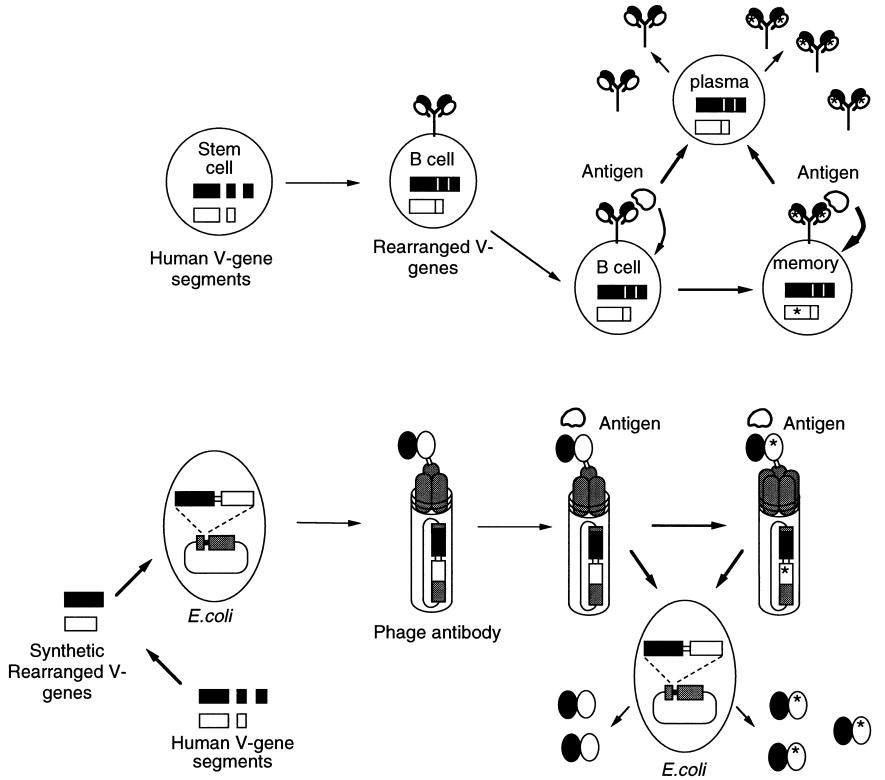

Fig. 1. A scheme adapted from [2] to compare the strategy of the natural and synthetic immune system for making antibodies.

body 'genotype' to 'phenotype', thereby allowing multiple cycles of selection.

Synthetic antibody gene repertoires have been cloned into filamentous bacteriophage to create phage repertoires of 10^7 to $>10^{10}$ phage clones [8,9,18]. The selection of such repertoires by binding of the phage to a range of different antigens has usually led to the isolation of phage with antigen binding activities. Indeed phage has been isolated that bind to protein antigens and to haptens, to foreign and self- (human) antigens and to highly conserved antigens; the binding is highly specific to the selecting antigen.

Soluble and folded antibody fragments can be produced by secretion from bacteria [19,20]; by introducing an amber stop codon between the scFv genes and the p3 protein, the fragments are displayed on phage produced from infected amber suppressor strains of bacteria, or secreted into the periplasm of infected non-suppressor strains [16]. Antibody fragments isolated from synthetic phage repertoires have been used for immunocytochemistry and for Western blots [18]. However the binding affinities were only moderate (10^5–10^6 M^{-1}). Improved binding affinities can be obtained by using larger repertoires (with greater diversity) [9] or by mutation of selected clones.

3. Mutation

For the affinity maturation of the synthetic antibodies it is necessary to introduce mutations, and then to select between phages displaying antibodies differing only slightly in binding affinity. Random mutations can be introduced in a variety of ways; for example by error-prone PCR [21], or by growth of the phage in a bacterial mutator strain [22]. The rate of mutation in the mutD5 strain is about 1/2000 bp, with transitions favoured over transversions, much as in somatic mutation in vivo.

For selection, it is possible to take advantage of differing dissociation rates of the phage from antigen. If two phages are bound to a surface that is then washed, the phages with slower dissociation rate are preferentially retained. As the dissociation rates follow an exponential decay, small differences in the dissociation rates can, over long wash times, lead to large enrichment factors [23]. By many cycles of mutation and stringent selection, it was possible to improve the binding affinity of a human antibody against a hapten by two orders of magnitude [24]. For selection of high affinity interactions it is also desirable to avoid the effects of multivalent interactions between the antibody fragments displayed on the phage and the solid phase antigen. The use of a phagemid to encode the antibody fused to p3, and a helper phage, favours the display of less than a single antibody fragment, and thereby is minimising avidity effects in the selection process [25].

In the immune system, mutations are also focussed at 'hotspots'; these tend to occur in antigen binding loops, in particular the first hypervariable loop of human VH and Vκ genes. With synthetic antibodies, mutations have also been focussed in loops using site directed mutagenesis [27,28] and this has led to dramatic affinity improvements and antibodies with nanomolar (and sometimes even picomolar) binding affinities. Such antibody fragments have been used for tumour imaging [28].

It therefore appears that by following what we know of the strategy of the immune system and using many of the same building blocks it is possible to make synthetic antibodies of predefined specificity and with useful binding affinities.

4. Repertoire design

The availability of an artificial immune system opens up further opportunities to understand the strategy of the immune system and to improve further our approaches to making synthetic repertoires.

Perhaps one of the areas of greatest potential is that of repertoire design. For example, although the immune system uses many different V gene segments, the structural diversity is much more limited, as the segments cluster into families of related sequences, and utilise loops with a limited number of folds [29]. Some segments are used preferentially; furthermore the same folds as represented in these segments also dominate the repertoire. The reasons for the domination of these segments and folds are not clear. The creation of synthetic repertoires based on these single folds may help to identify whether such repertoires are sufficient for recognition of the complete 'universe' of antigens.

A further aspect of repertoire design is the location of sequence diversity. By comparing the sequences of rearranged antibody genes with those of the segment building blocks, it is possible to compile maps of sequence diversity in the antigen binding site before and after somatic mutation. The patterns of diversity are complementary; the greater the diversity in the primary repertoire, the less the diversity after somatic mutation (and vice versa) [30]. Presumably the mutation of a residue in a 'crude' binding site that already makes an intimate contact with antigen is more likely to destroy the interaction than the mutation of residues directly adjacent. Such features of 'complementarity' could be advantageously incorporated into the design of synthetic repertories.

5. Implications for other proteins

The creation of a primary antibody repertoire involves the assembly of structural elements with a common fold. Presumably other proteins could be engineered in the same way. Indeed the creation of gene repertoires by extensive recombination between related proteins appears to be directly analogous to the creation of the primary antibody repertoire (and indeed very similar to the generation of antibodies in birds which appears to use gene conversion) [31,32]. Likewise the evolution of proteins by mutation and selection is directly analogous to affinity maturation in antibodies [33]. Approaches that harness all the stratagems of the immune system for making antibodies would seem to have the potential for creating and refining proteins with a wealth of new activities.

Acknowledgements: I would like to thank Dr. Ian Tomlinson for discussions of aspects of repertoire design.

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