Heteropolyvalent Hybrid Antibodies of Enhanced Discriminatory Power. Applications In Oncology[1]

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ABSTRACT

Though not always successful, the immune system is certainly active against tumours, wherefore many attempts have been made to harness antibodies for the delivery of toxins or other effectors to cancer cells. The disappointing results are probably due to limitations of both tissue penetration and selectivity.

Discriminatory power can be magnified by linking together two or more binding units that are specific for distinct epitopes on the same target molecule or cell, though only if the linkers are flexible enough to allow all units to bind simultaneously without strain. A new specificity, of higher level, is created, namely to a constellation of epitopes rather than a lone star. To allow precise description, the Discrimination Constant, D, is defined as the ratio of affinity constants (of a complete ligand molecule) for a chosen target and a comparator. It is dimensionless and is conceptually distinct from affinity or specificity. A comparator must be identifiable explicitly or by implication.

Adducts can be made with a nucleic acid single strand attached to an Fab fragment (or a smaller, engineered entity) so that when adducts come together in vitro or in vivo they assemble spontaneously into a heteropolyvalent hybrid. Marker or effector entities can be incorporated in the same way. The epitopes to be attacked can be chosen on an individual basis and if appropriate adducts are available, ready-prepared, the desired hybrid is formed instantly merely by mixing them together.

Notes:
1 If citing this work, please give the full title if possible, and continue, 'Cell Signalling and Novel Cancer Therapeutics', Joint Meeting of the Oncology Section of the Royal Society of Medicine and the British Association of Cancer Research, London, UK, 29th November 2007 [poster P4].
2 'Enhanced Discriminatory Power from Polyvalent Binding. Specificity, Selectivity, Discrimination, Molecular Flexibility'. Personal Communication, T.R.C. Boyde, 2007
Both texts will be available on the following website: www.trcboyde.net

INTRODUCTION

It is a mistake to think that natural antibodies show particularly high affinities. The highest are exceeded up to a million fold by those optimized in the laboratory and by avidin, also a protein, subject to the same physical principles and employing the same amino acids; so that limited binding power is not due to any shortcomings of the molecular tools at hand. Rather, it has evolved to be like that. Likewise, the specificity of antibody recognition is neither absolute nor even very impressive – if by that we mean the discriminatory power of the individual binding site (Fab).

What **IS** remarkable about natural antibodies is that they are always polyvalent, and one by-product of the present enquiry is clearer insight as to why that should be so. It will appear that a polyvalent antibody shows not only greater affinity for its polyvalent target, but also can discriminate for that target even against a background containing the monovalent epitope in abundance. This applies just as much to natural, homopolyvalent antibodies, but comes more

easily to the mind in connection with artificial constructs, which have additional potential uses and specificities of a new order.

MOLECULAR REQUIREMENTS FOR HIGH AFFINITY

A binding site that neatly fits the target allows more atoms to take part in contacts favourable for binding and the same applies to a larger binding site or a combination of binding sites (Figure 1). Neat fit requires more than just complementary shapes: charge, the nature of atoms and orbitals, solvent molecules, all come into play: and any strain in either partner takes up part of the energy available from the interaction so that net binding energy and therefore affinity is diminished (Figure 2).

SELECTIVITY VERSUS SPECIFICITY

The word 'specificity' has more than one meaning – perhaps a whole family, certainly at least two distinct senses. It helps to use a different word for the quantitative aspect (X binds more strongly to U than to V), write this as 'selectivity' or 'discriminatory power', and define a discrimination constant, D, as the ratio of the affinity constants. Then if ligand Y is a better discriminator, that is to say better than X at distinguishing U from V, this appears as a greater value of D for the same two targets. A comparator such as V must always be in mind, or else is implied and we call it 'background'. A basically similar theory in terms of avidity deals with rank order rather than continuous algebraical functions.

HETEROPOLYVALENT ANTIBODY CONSTRUCTS

Figure 3 shows some of the kinds that have been around for a while; the first, recombined chains of natural antibodies, for 40 years; chemically combined Fab's for almost as long; genetically engineered diabodies and the like for 15 years. Their use has nearly always been to tie together two independent targets as shown in Figure 4, so that the targets are brought into physical proximity, e.g. for labeling in histochemistry or occasionally with therapeutic intent. Perhaps because of a feeling that the level of specificity and affinity available from natural Fab binding sites must be good enough, there has been little or no interest in combining specificities for different epitopes on the same target, and yet it is commonplace that a cell surface or even an individual protein molecule may exhibit many different epitopes. The kind of thing that might happen is shown in Figure 5. Here we can say that a new specificity has been created; the hybrid is specific for the presence of both epitopes simultaneously; but the affinity of the interaction is diminished by the strain involved in bringing two Fab's into contact with the target at the same time.

Though 'Fab' is used here as a kind of shorthand, we are certainly not confined to using the natural product; better and smaller engineered products are or will be available.

FLEXIBLE LINKERS

The strain problem may be solved by the use of a flexible linker, as in Figure 6.We are left to worry about possibly losing tightness in the overall binding reaction because the ligand assembly is too floppy. All one can say at the moment is that the extent of this loss has been exaggerated by some, and especially so if the linker is composed of peptide or nucleic acid.

CONSEQUENCES FOR AFFINITY AND SELECTIVITY

It is certainly not true that the affinity of a polyvalent construct ought to be exactly predictable by multiplying together the affinity constants or adding the binding energies of individual Fab's: nevertheless there is ample reason, from published results reviewed briefly in the accompanying paper[2], to expect very large enhancement. A theoretical approach on the basis

of ‘selectivity’ and the discrimination constant makes it easier to handle the consequences as to discrimination and specificity. For example, using very modest exemplary affinity figures, a heteropolyvalent hybrid might have affinity (K_d) of 10^{-12} mol/l for its heterobivalent target, 10^{-7} mol/l for each of the component epitopes, giving D values of 10^5 in each case. In principle, the hybrid can pick out the combined epitopes, present together on a single target, even in an environment exhibiting those same epitopes present separately on cross-reacting targets, whether as monovalent entities or in homopolyvalent form.

A novel specificity has been created, for the heteropolyvalent target. This is a specificity that did not exist until the hybrid was made.

Of greater practical importance is that the heteropolyvalent target can be distinguished from other forms of matter in a new way, as a new species of matter, and that the method is extendable in principle to any epitopes for which Fab’s or other binding groups can be devised and in any combination.

The natural homopolyvalent antibody must exhibit the same effects in its mode of action, which lets us see more clearly that it is specific for a polyvalent target, and that this is a specificity distinct from and additional to the specificity of the individual Fab on its own. Evolution has selected for a modest affinity of the monovalent reaction: the polyvalent principle meant that nothing more was needed, or may have proved disadvantageous.

NUCLEIC ACID LINKERS

It turns out that nucleic acid linkers are neat and advantageous:-

1] Adducts with an oligonucleotide tail (Figure 7) are easily made,
2] may be of fairly low molecular weight, e.g. about 20kDa,
3] readily self-assemble forming a link that is as stable as necessary,
4] having also a flexible segment that is as long as necessary (Figure 8).

Even more advantageous:-

5] Hybrids can be made with >2 Fab’s,
6] plus additional effector or marker components (Figure 9),
7] in a few moments (given stock of the necessary adducts),
8] tailored for the individual case,
9] even within the body, on site, *in vivo*,
10] thus avoiding problems of tissue penetration,
11] and may eventually provide for intra-cellular access.

APPLICATIONS IN ONCOLOGY?

We classify cancers and treat accordingly, but as time goes by it is more and more clear that the detailed mechanisms of a particular cancer are in large part independent from how it may happen to have been classified. We need better tools for distinguishing the constellation of characterising features or epitopes of one tumour (or one clone within a tumour) from another. The hybrids discussed here apparently provide a route to this goal, since the affinity and discriminatory power can be ramped up indefinitely. For diagnostic purposes the functional component would be an appropriate marker. For treatment, a grouping of Fab’s identified at the diagnostic stage would be combined with a cytotoxic effector component.

This discussion reflects a relatively crude model of cancer biology and the potential for applications in treatment: restraint of discordant cells may eventually prove to be the best approach in all cancers as it already is in some. And yet, would not any patient prefer to know that all his cancer cells, at least all those of detectable and definable genotypes, had been eliminated? Is it worth a try?

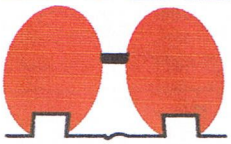
Figure 1

different specificities homobivalent target

Figure 2

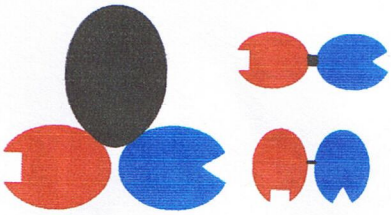
badly fitting less affinity

epitopes too far apart



Heteropolyvalency

Figure 3



'diabody' connecting two distinct targets

Figure 4

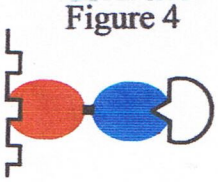


Figure 5

binding with distortion

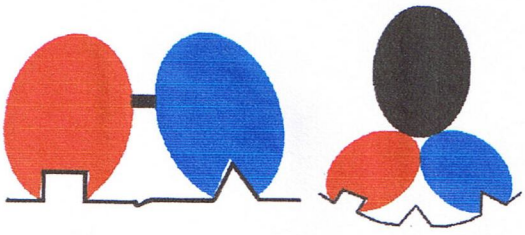
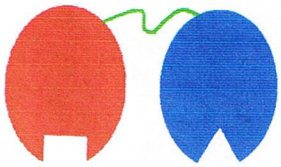


Figure 6

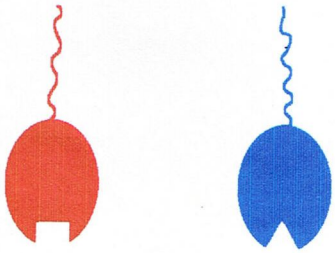
Flexible linker



Nucleic acid linkers

Figure 7

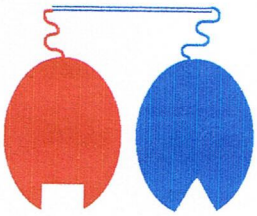
'adducts with single-strand DNA tails



Nucleic acid linker

Figure 8

duplex forms between complementary segments



Nucleic acid linkers

Figure 9

three or more AB units, no problem

add functional components, no problem