

## Discovery of homophilic antibodies as novel drugs

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### ABSTRACT

In 1986 we discovered that a murine monoclonal antibody, TEPC15 (T15), could bind to itself through homophilic affinity. Subsequently we located the region in T15 that is responsible for homophilic binding. Homophilic antibodies are infrequent as of today only four monoclonal homophilic antibodies have been described, but we found that homophilic polyclonal antibodies are in serum of normal mice and humans. The homophilic activity resides at the CDR3-FR4 region of the T15 heavy chain and a peptide of this sequence can confer the homophilic property to other antibodies using chemical and recombinant technologies. *In vivo* and *in vitro* studies have shown that men-made homophilic antibodies are more potent than their corresponding native antibodies. These findings suggest that homophilic antibodies could become a novel class of highly effective therapeutic drugs.

**KEYWORDS:** antibodies, polyvalent, homophilic, immunotherapy

### INTRODUCTION

Homophilic or self-binding antibodies are a new class of antibodies that were only recently discovered. Such antibodies bind to targets as polyvalent antibodies with increased valency and potency over conventional bivalent antibodies. In the following I recount the discovery of homophilic antibodies and describe approaches how to make and use them.

### Natural homophilic antibodies

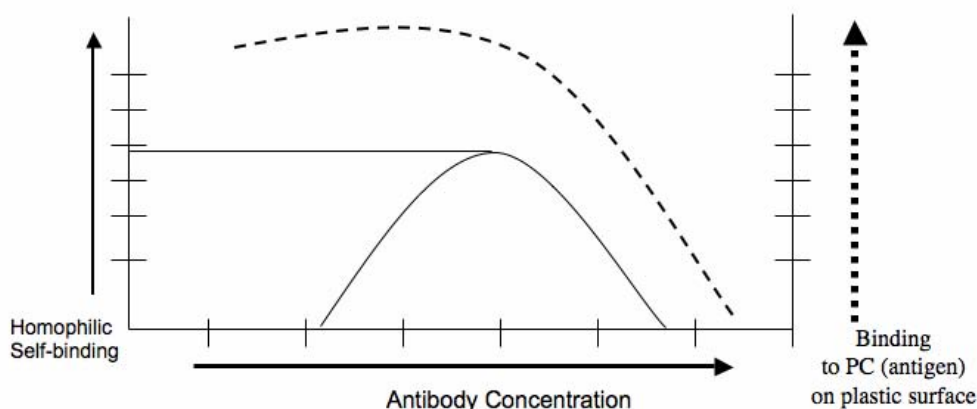
During the 1980s I was involved in studies on the regulation of B and T-cell immunity via idiotypic networks [1] and developing idiotype-base vaccines [2]. As a part of an experiment to characterize monoclonal antibodies against phosphorylcholine (PC) in solid-phase radioimmuno-assays, I asked my graduate student, C.-Y. Kang, to include in the experiment the TEPC-15 (T15) anti-PC monoclonal antibody as a control. To our surprise, radio-labeled T15 bound to the insolubilized T15. I resisted the temptation to disregard this unanticipated result as mere artifact. We interpreted this unexpected finding as being the consequence of an internal image idiotype within the T15 that mimics the structure of the PC antigen [3]. Such self-binding, or homophilic, antibodies could represent a mini-network important for self-regulation, and functionally control the dominant expression of the T15 idiotype in Balb/c mice [4].

Figure 1 shows the data that led to the discovery of homophilic antibodies. Note that the self-binding effect shows a concentration dependent bell-shaped curve.

The year 1986 marks the beginning of a long slow process to understand the phenomenon of homophilic antibodies. We continued to look for other antibodies against PC that might be homophilic, isolating and comparing a monoclonal hybridoma anti-PC antibody of the IgM class to IgA and IgG anti-PC antibodies. The pentameric IgM anti-PC antibody displayed a much higher self-binding property than the IgA or IgG antibodies [5]. We inferred that homophilic

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**Figure 1.** T15 antibody was insolubilized on plastic wells, dilutions of labeled T15 were added and binding measured (left X-axis). PC antigen was insolubilized on other wells and binding of labeled T15 antibody was recorded (right Y-axis). (Taken from: C.-Y. Kang and H. Kohler, *Annals New York Acad. Sci.* 1986).

binding in solid-phase assays was augmented by the polyvalency of the homophilic antibody.

Our next breakthrough came when we approached Ed Blalock to analyze the T15 Vh and VJ sequences for internal hydrophathy motifs that might explain the homophilic nature of the T15 antibody. A sequence region in the T15 Vh was identified as having inverse hydrophathy, and a peptide was made representing the amino acid sequence 50 to 74 of the T15 Vh, comprising parts of the CDR2 and FR3 region. This peptide inhibited the self-binding of T15, while control peptides had no effect on the homophilic interactions of T15 [6]. This finding identified the homophilic domain of the T15 structure, in which the predicted homophilic attraction resided in a looping sequence, whereby the region 50-60 interacts with the sequence 63-74 in an anti-parallel manner.

In 1989 Srinivasa Kaveri, a young postdoctoral fellow from the Kasatchkine Lab in Paris France moved with me to IDEC Pharmaceutical in San Diego. Srinivasa became very interested in homophilic antibodies, and started to work on the state of the homophilic T15 antibody in solution. We speculated that homophilic interactions may form complexes in solution, yet at a very low level, since we never observed precipitation of the T15 antibody with concentrations as high as 5 mg/ml. When T15 was dissolved in a solution that was 10-fold lower than physiological ionic strength, Ig complexes could be detected by gel filtration; also

phosphate buffered saline containing 2% PEG revealed higher molecular species of T15. Evidently, an equilibrium existed between the monomeric and dimeric forms of T15, an equilibrium that was markedly shifted under physiological conditions to favor the monomeric form [7].

What we knew thus far was that T15 was a common germline antibody in mice, its anti-PC response was idiotype dominant in Balb/c mice [8], and it exhibited homophilic properties [4]. It was therefore logical to look for “natural” antibodies in mice that would bind to the homophilic T15 domain (Vh 50-74). Such antibodies recognizing the homophilic peptide were present in normal mice sera, and, to our surprise, in sera from normal human donors [7]. Our interpretation today is that binding of natural antibodies in mouse and human sera is due to the homophilic interaction between the homophilic domain in antibodies and the insolubilized T15 peptide.

Roberta Halpern coming from Don Mosier’s lab in San Diego had joined my lab at the La Jolla Cancer Research Foundation. She embarked on the search of the T15 anti-PC antibody in human sera. It was tedious work with multiple columns and ELISAs, testing both her patience and mine. A tragic event interrupted our work, as Roberta was killed attempting to rescue a woman who had fallen onto the tracks as a train approached the station in Del Mar. At that time Roberta had

**Table 1.** Homophilic monoclonal and polyclonal antibodies.

Antibody	Antigen	Year discovered	Reference
T15/S107	PC	1986	Kohler <i>et al.</i>
R24	GD3	1990	Chapman <i>et al.</i>
Polyclonal Human/mouse	PC	1991	Haplern <i>et al.</i>
D7.4	ssDNA	1992	Voss <i>et al.</i>
4G3	Digoxin	1993	Tyutyulkova <i>et al.</i>
4B8	TPO	1999	Duthoit <i>et al.</i>

produced enough data so I could write a manuscript and publish her work posthumously [9].

Other examples of homophilic Ig were independently reported by groups at Sloan Kettering, NY [10] and the Université Méditerranée, France [11]. Interestingly, the homophilic domain in the anti-GD2 antibody, called R24 [10], comprises the same CDR2-FR3 region as in the T15 antibody. As of today four monoclonal homophilic antibodies against different antigens are known as well as polyclonal homophilic antibodies against PC have been described in men and mice (see Table 1).

It is striking that these naturally occurring homophilic antibodies are directed against non-protein antigens. Non-protein antigen cannot engage the T help needed to switch Ig classes and to increase their affinity through rounds of mutation and clonal selection. Thus T-independent antigens generally induce low-affinity IgM class antibodies that compensate for low affinity with increased avidity of the IgM pentameric structure. Another of nature's way to deal with non-protein antigens is the homophilic activity that also increases avidity by allowing polymeric targeting. The reason why homophilic antibodies appear to be a rare species lies in the limitation posed by incorporating the homophilic domain into Ig molecule without affecting binding and affinity.

I asked Betty Diamond from the Albert Einstein Institute, NY to send me the T15 mutants U4 and U10, that had lost binding to PC, but gained affinity to dsDNA [12]. These T15 mutants still

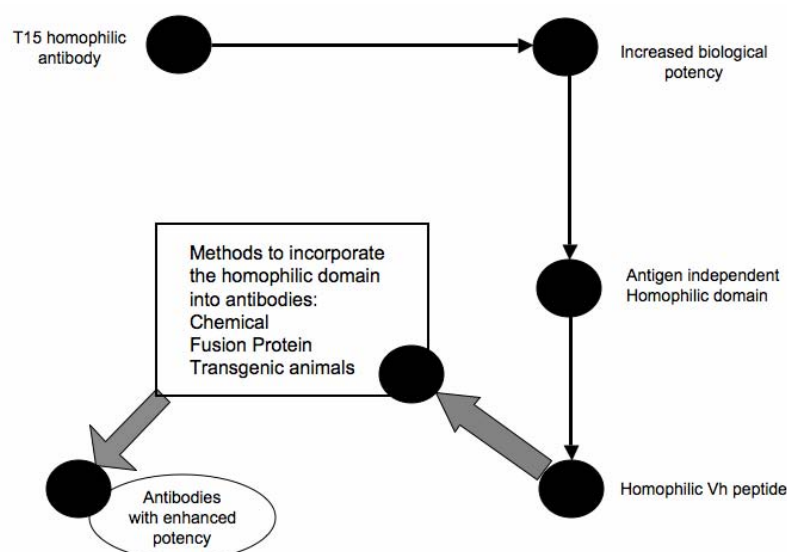
expressed the T15 canonical idiotype, and, when we tested for homophilic binding, we found that both the U4 and U10 antibodies were homophilic. We then concluded that there were two classes of antibodies: the common and major type, and a rare class that exhibits homophilic character. It showed that homophilic antibodies can have different antigen specificities, thereby increasing antibody diversity [13]. This work also implied that the homophilic property could be added to any antibody without changing its antibody specificity and affinity.

In 1991 I left IDEC and took a position at the Markey Cancer Center of the University of Kentucky. Charged now with the mission to develop better cancer therapies, I remembered an earlier work by L. Claflin, who had compared the protection against pneumococcal infection imparted by anti-PC antibodies of different idiotypes. The T15/S107 anti-PC provided thousand-fold better protection against the bug than the MOPC167 or 603 anti-PC antibodies that have an identical affinity for the PC hapten [14]. Based on the superior potency of T15 idiotype antibodies, I connected these dots (see Figure 2).

### Engineered homophilic antibodies

Based on this line of thinking, I hypothesized that the presence of the T15 peptide within a given antibody would confer homophilic character upon that antibody and make it more potent biologically and immunologically.

What was needed to test my hypothesis was a method to incorporate the T15 homophilic domain



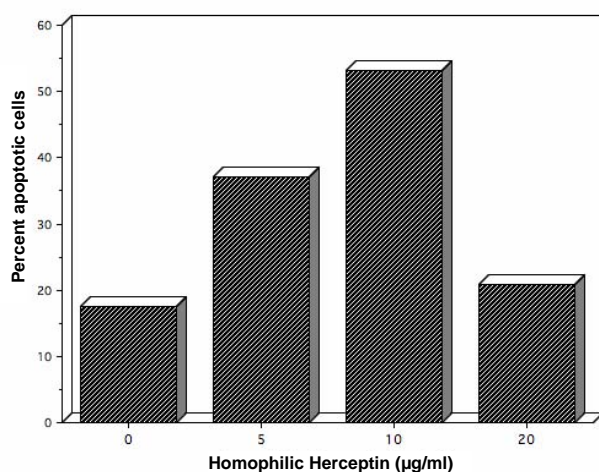
**Figure 2.** Flowchart of key conceptual and experimental milestones leading to developing homophilic antibodies with enhanced potency covering a time span of 22 years.

into an antibody without changing the antibody's antigen specificity, affinity, and biological effector functions (see Figure 2). The method I dreamed of should be simple, efficient, and specific for antibodies. I needed help from a chemist with experience in protein affinity crosslinking. Fortunately, Alan Kaplan, the chair of Microbiology/Immunology at the University of Kentucky, connected me with Boyd Haley, an expert in photo-activated affinity labeling methods. Boyd showed me a gel with  $^{32}\text{P}$  azidoATP and immune precipitated ATPase. The gel had two bands which looked to me like heavy and light chains of antibodies, and so it dawned on me that this might be the method I needed for affinity labeling antibodies. I gave Boyd some monoclonal antibodies for his photo-activating ATP labeling. The gels clearly showed two bands with the molecular weight of heavy and light chains. This started a productive collaboration with the Haley lab, leading to the discovery of an unconventional ATP binding site on antibodies [15]. Gabriela Pavlinkova was a major player in the subsequent work, firmly establishing the presence of a nucleotide affinity site on antibodies and antibody fragments of different specificity and origin [16].

It appeared that I had now a method to connect the next dots in the homophilic puzzle (see Figure 2): to

affinity crosslink the homophilic T15 peptide to antibodies and to show an increase in functional potency (as nature did with the potency of the T15 antibody against pneumococcal infection) [14]. We chose CD20 as a target, given that IDEC's Rituxumab has become a blockbuster therapeutic antibody because it would be very exciting if the crosslinking technology could improve antibodies potency against CD20. In 2002 we published two manuscripts [17, 18] that provided proof of principle: chemical conjugate antibodies with the homophilic domain have a higher potency than their naked antibody counterparts. Moreover, it was reassuring to demonstrate that the in-solution monomer/dimer equilibrium of T15 peptide conjugate antibodies was similar to the in solution equilibrium of the monomer and dimer forms observed with the natural T15 antibody. These dimerizing antibodies showed enhanced targeting and inhibition of growth of tumor cells *in vitro*, and strikingly greater induction of apoptosis than the naked antibody. Subsequent studies with T15 peptide-conjugated anti-Her2/neu antibody (Genentech's Herceptin) in a mouse xenograft tumor model showed superior suppression of tumor growth relative to naked Herceptin [19].

While working with homophilic Herceptin targeting a human tumor cell line we observed a



**Figure 3.** Apoptosis in 1650 human lung cancer cells was induced with different concentrations of homophilic Herceptin. Percent of apoptotic and pre-apoptotic cells are shown (data taken from [19]).

variable potency in binding and apoptosis assays. The amount of induced apoptosis was the highest with an intermediated dose of homophilic Herceptin. As seen in Figure 3, 10 µg/ml of antibody induced more apoptosis than 5 µg or 20 µg. This concentration depended response optimum resembles the bell-shaped curve of homophilic binding seen in Figure 1. If this dose-dependent response holds true in *in vivo* studies, therapies with homophilic antibodies need to be tailored with respect to tumor burden in patients.

In 2002 InnexusBiotech, a start-up biotech company in Vancouver, Canada, acquired the rights from Immpheron to the affinity crosslinking technology (US patent #6, 238, 667). Based on this intellectual property, Innexus attracted investors to exploit the technology. Another demonstration of the value of the T15 technology was the rescue of a humanized antibody, which we obtained from a biotech partner [20]. As humanization occasionally leads to the loss of activity, homophilic conversion offers the possibility to restore activity the humanized antibody has lost.

The initial protocol of photo-affinity crosslinking utilized an azido-peptide or an azido-nucleoside-peptide construct. A simplified method was developed that avoided the use of an azido compound, as we found that the amino acid

tryptophan can be crosslinked to antibodies without affecting the specificity or affinity. Peptides that contain an end-terminal tryptophan also crosslink to antibodies. The kinetics of this crosslinking suggests that, in the immunoglobulin structure, there are affinity pockets for photo-inserting tryptophan side chains [21]. While the chemical methods of crosslinking biologically active peptides, including trans-membrane peptides [22], is simple and effective, a recombinant version of homophilic antibodies would be preferred for large scale production of therapeutic antibodies.

### Past and future

Homophilic antibodies once occurred only in nature, but now can be produced at-will. Recounting the history of homophilic antibodies still puzzles me as it took more than 20 years to recognize their potential, as well as to find ways to make such superior antibodies. The relevant data had been produced, in part wrongly interpreted, and lay unconnected for a long time. In retrospect it should have been obvious after the discovery of homophilic antibodies that this feature could be exploited, but history shows that often the human mind moves slow, both in being receptive to novel facts and/or biological properties, and in changing thinking. There still remains research to be done to fully exploit Nature's of putting a homophilic domain into antibodies. For example, we [4, 23] and others [10] observed that the self-binding titration is not linear, but follows a bell-shaped pattern whereby the high and low concentration show no self-binding. This predicts that a therapeutic concentration window exist that needs to be understood.

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