The Impact of the Hybridoma Technology on the R&D of Idiotypic Antibodies

Heinz Kohler

The PubMed data set was scanned with the title and abstract term “Idiotypic” followed by secondary searches with “Vaccine” and “Clinical trial.” The retrieved references were analyzed from the period before and after hybridoma technology (1975). In 1963, Oudin and Kunkel discovered that antibodies against antibodies can be raised to identify determinants unique to an antibody termed idiotype or individual antigenic determinant. Two laboratories reported that anti-idiotypic antibodies can suppress specific antibody responses in mice. In 1974, Jerne proposed a network of idiotypes and anti-idiotypes and the functionality of the idiotype network was confirmed. This prompted the proposal of a symmetrical regulatory immune response. By 1989, the concept and the functional parameters of the immune idiotype network were established in the prehybridoma period. It was not until 1981 that monoclonal anti-idiotypic antibodies were used as tools to study the expression of idiotypic determinants on antibodies and to categorize functional properties in the immune network as network antigens in 1989. Hybridoma-generated monoclonal anti-idiotypic antibodies provided the tools to precisely identify different idiotypic regions on antibodies and test these as targets to induce network cascades. The initial distinction of Ab2s as alpha and beta were expanded to include gamma and delta. The initial concept of Ab2beta being an antigen internal image, used as vaccine, was challenged showing that targeting all idiotopes on B cell receptors can induce specific antibodies. After the discovery of the hybridoma technology a wave of idiotype topic publications occurred, that declined by 2015. In 1985, in this wave of reports on anti-idiotypes, their importance to vaccines dominated. These vaccines targeted in animal models parasite, bacterial, and viral diseases, and cancer. The reported data indicated a therapeutic response in inbred mice. The issue of idiotype matching between mouse haplotypes of vaccine origin and treated mice were raised. In 1995, the human clinical trials in different cancers using anti-Id vaccines were reported. Only one such vaccine received conditional approval in Argentina and Cuba, whereas the other trials failed in phase II and III. The reasons for this failure were subsequently discussed. Although the use of the Milstein and Kohler hybridoma technology and subsequently alternative methods to produce monoclonal animal and human antibodies created a new class of drugs, commonly referred as “Biological,” it failed on the promise therapeutic of anti-Id vaccines.

Keywords: idiotypic antibodies, human clinical trials, hybridoma technology

Introduction

THE DISCOVERY of monoclonal antibodies was facilitated by NATURE with the disease of multiple myeloma. Waldenstrom described a bone marrow tumor that produces a macroglobulin (IgM).⁽¹⁾ Bence-Jones protein in urine of multiple myeloma patients was another “natural” source of monoclonal Ig light chain.^(2,3) Subsequent reports showed the primary and three-dimensional structure of immunoglobulins.⁽⁴⁻⁶⁾ Whereas these structural studies were done on immunoglobulins from human B cell tumors, Potter developed a method to induce plasmacytomas in inbred mice that exhibited antigen binding.⁽⁷⁾ The study of a group of mouse monoclonal antibodies with

specificity for phosphoryl choline and a shared idiotypic specificity marked the beginning of intensive idiotype investigations.⁽⁸⁾

The Time Before the Hybridoma Technology

To appreciate the impact of the hybridoma technology on the field of idiotype one should describe the landscape of idiotype R&D at the time before Kohler and Milstein.⁽⁹⁾ The primary task of antibodies is providing immune defense against pathogens. Thus, why would Nature turn antibodies against each other? The mechanism of antibody against antibody implicit in idiotype network appears unnatural. And why would such activity be involved in the regulation of immune response?

Department of Microbiology and Immunology, University of Kentucky, Lexington, Kentucky, USA.

Hints of immune homeostasis were found as early as 1964 by Rowley and Fitch.⁽¹⁰⁾ Passive injection of homologous anti-sheep erythrocyte serum inhibited a primary antibody response. The authors conclude that the formation of specific antibody may provide a homeostatic or “feedback” mechanism affecting the B cell receptor (BCR) being itself antibody.

The discussions of anti-antibodies started in 1957⁽¹¹⁾ and continued in 1964.⁽¹²⁾ Oudin and Michel^(13,14) studied anti-allotropy in rabbits and discovered a new type of anti-antibody that he termed anti-idiotypic. It should be noted that none of these anti-idiotypic antibodies could be induced in the rabbit that was immunized to produce anti-idiotypic antibodies. Thus, these anti-idiotypic antibodies are not autoantibodies.

Although these conceptional studies continued, much progress was made in the field of immunoglobulin structure of multiple myeloma proteins, so-called paraproteins.^(2–5,15–18) Potter devised a method to induce plasmacytomas in Balb/c mice with antigenic specificity.⁽¹⁹⁾ That provided a new source of monoclonal antibodies for structural and three-dimensional studies.^(20,21) Among these Balb/c mouse monoclonal antibodies was a group of antibodies against phosphorylcholine with shared idiotype specificity.⁽⁸⁾

In 1973, Jerne⁽²²⁾ speculated on a network of interacting lymphocytes and antibodies, which he expanded in 1974.⁽²²⁾ The immune network theory became very popular creating a wave of anti-Id R&D (Fig. 1). Experimental support was obtained by demonstrating an auto-anti-Id response after an immune response.^(23,24) These findings let to the proposal of a symmetrical immune response of Id and anti-Id.^(25,26) By 1975, the theoretical and experimental aspects in the idiotype landscape were completed.

The Time After the Hybridoma Technology

The new technology of making monoclonal antibodies against selected targets⁽⁹⁾ boosted the work on idiotypes (Fig. 1). It was now possible to generate monoclonal anti-idiotypic antibodies. With these new tools the individual idiotypic determinants (idiotope) were dissected and the topography and network function of antibodies could be studied.^(27–30) Interestingly, monoclonal anti-Id blocking the antigen binding (Ab2alpha) and not blocking monoclonals (Ab2beta) stimulated anti-phosphorylcholine B cell⁽³¹⁾ challenging the “internal image” concept of Jerne.⁽³²⁾

Although the basic research on idiotype network continued, a new aspect emerged in the field of immunotherapy. Supported by the reports that anti-Id antibody can suppress B cells,⁽³³⁾ Levy and colleagues⁽³⁴⁾ prepared a monoclonal anti-Id against a patient’s B cell tumor and used it to suppress the idiotype expressing tumor. This approach led to a new concept in immunotherapy⁽³⁵⁾ and to a startup Biotech company devoted to passive and active anti-Id therapy (IDEC Pharmaceuticals), which eventually brought the first cancer-directed chimeric antibody Rituxan to market. The hybridoma technology was quickly exploited to produce monoclonal anti-idiotypic antibodies as so-called internal antigen images (Fig. 2). Monoclonal anti-Id antibodies of the Ab2beta type were tested in animal models against viral and bacterial infections and cancers.^(36–42) These anti-idiotypic experiments in animals did induce antigen-specific responses and vaccine-induced protection was also observed.

The positive results in animal models were encouraging to use cancer targeting anti-Ids in clinical trials (Fig. 3). Several reports on human trials with monoclonal anti-idiotypic antibodies

FIG. 1. Plot of a PubMed search with the search terms idiotype and anti-idiotypic. The area under the line represents the search return at given year. The year of the Milstein/Kohler publication is indicated as well as the year of an anti-Id vaccine approval.

FIG. 2. The data of search in Figure 1 were search with the term of anti-Id vaccine. Each bar represents the number of vaccine experiments per year.

appeared between 1992 and 2018 (Fig. 3). The patient's response in these trials has been recently reviewed.⁽⁴³⁾ Only one monoclonal anti-Id vaccine was approved as drug against tumors in Cuba and Argentina. It was argued that the failure of anti-Id vaccines in human trials is due to the narrow and mismatched idiotypes between the origin and vaccine target idio-⁽⁴⁴⁾ type. This conclusion is supported by data reported by Brown and Sealy.⁽⁴⁵⁾ The authors generated five monoclonal anti-idiotypic against a dominant anti-p-phenylarsonate (ars) monoclonal. Each anti-Id did not compete in binding to the anti-ars monoclonal. Furthermore, each anti-Id suppressed on a portion of the total anti-ars response indicating a multitude of different idiotypes in the anti-ars response of different strains of mice. In contrast, in previous experiments⁽⁴⁶⁾ complete suppression of the anti-ars response was achieved with polyclonal anti-idiotypic antibody. Realizing the diversity of idiotypes in the BCR repertoire responding to a given antigen effective stimulation would require an equally diverse anti-Id pool. Thus, the proposal was made to target multiple idiotopes on BCR using pools of polyclonal anti-idiotypic antibodies as vaccine.^(47,48)

FIG. 3. The data in Figure 1 were search with the term human anti-Id trial.

Conclusion

At the time the hybridoma technology emerged most of the functional and theoretical aspects of the idiotypic paradigm had been known. The means to produce monoclonal antibodies was quickly realized to produce monoclonal anti-idiotypic antibodies. These were used to study the topography of idiotopes on antibodies and to dissect antigen-induced network responses. The major impact of the new technology was the field of therapeutic idiotypic vaccines, based on the “internal image” concept. The induction of specific antibody response using monoclonal anti-idiotypes was documented in animal models. However, idiotypic-based therapeutic vaccines failed in the clinic except for one in South America approved. An emerging change of the understanding the idiotypic network led to the proposal of abandoning monoclonal antibodies and using polyclonal idiotypic vaccines.

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Address correspondence to:

Heinz Kohler, MD, PhD

Department of Microbiology and Immunology

University of Kentucky

5235 Athens Boonesboro Road

Lexington, KY 40509

USA

E-mail: hkohl00@uky.edu

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