Monoclonal antibodies as blood grouping reagents

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Introduction

It is a great privilege to participate in this symposium to honor the career of Dr. Peter Issitt. It has been my good fortune that Peter has been both a personal friend as well as an esteemed professional colleague for more than 30 years. This 30-year period has been highlighted by the many contributions made by Dr. Issitt to the field of immunohematology. It strikes me as especially fitting that he should mark his retirement with the publication of the fourth edition of Applied Blood Group Serology, which, like its predecessors, will become the standard text for this field. A significant portion of chapter 7 is a discussion of the impact of monoclonal antibodies, the subject of my presentation.

A relatively seamless transition from human polyclonal to monoclonal sources of blood grouping reagents occurred in the last decade, facilitated largely by two independent events. The first was the declining availability of injectable blood group substance for hyperimmunization programs; the second was the simultaneous emergence of hybridoma technology. The transition to monoclonal sources of routine blood grouping reagents began shortly after the report of Köhler and Milstein in 1975. Currently, virtually all commercially prepared ABO grouping reagents are formulated from murine monoclonals. It is clear that reagents for D typing will soon follow suit. Monoclonal reagents have been well accepted although a few unexpected complications have been recorded, mainly during ABO grouping. Other concerns about the restricted specificity of monoclonal anti-D reagents might require a reappraisal of traditional approaches to D typing. These issues will form the basis of this presentation.

ABO Grouping Anomalies

The first monoclonal ABO blood grouping reagents were offered in 1985 by Immucor, Inc., Norcross, GA. Later that year, Ortho-Clinical Diagnostics, Raritan, NJ, launched the BioClone™ range of murine monoclonal reagents. These soon proved to be as effective as traditional polyclonal reagents; in fact, BioClone™ anti-A exceeded the ability of human polyclonal anti-A to detect weak subgroups of A to an extent that the continued need for the routine use of anti-A,B was questioned. However, this extra sensitivity soon proved problematic.

The B(A) phenotype

Some early users of BioClone™ anti-A encountered anomalous reactions with group B cells. Beck and coworkers observed that nearly 1 percent of group B donors in Kansas City were reactive with BioClone™ anti-A. MH04, one of the two clones from which this reagent was formulated, was ultimately shown to be responsible for these spurious reactions. MH04 was selected by the manufacturer because of its ability to agglutinate red blood cells (RBCs) belonging to weak subgroups of A. Beck and colleagues concluded that MH04, capable of directly agglutinating many examples of A cells, also was able to detect the very sparse A sites that exist on eclectic group B cells. This phenomenon, which came to be styled the B(A) phenotype, was shown by Beck et al. to be directly associated with elevated levels of serum D-galactosyl transferase. Individuals belonging to the B(A) phenotype were found to have sufficiently elevated levels of the B-gene-specified transferase to permit the transfer of the A-determining sugar, N-acetyl-D-galactosamine, as previously demonstrated by the in vitro studies of Greenwell et al. The detection of B(A) appears to have been eliminated by reformulation of the reagent and is included in today’s discussion only to serve as an example of a problem totally unanticipated by premarketing trials. It is reasonable to expect that other new products may be similarly bedeviled.

The A(B) phenotype

Because Yates and Watkins had earlier demonstrated the biosynthesis of blood group B determinants by the A-gene-specified transferase in vitro, it was reasonable to anticipate the agglutination of group A cells by potent
examples of monoclonal anti-B. Thus, it was no surprise when Voak et al. reported that a potent monoclonal anti-B (BS85) had the capacity to weakly agglutinate the RBCs of about 1.5 percent of group A donors. The reaction could be specifically inhibited by group B secretor saliva, but unlike B(A), was not related to excessively high levels of serum A-transferase. This phenomenon was called the A(B) phenotype. No further examples of either B(A) or A(B) have been reported with commercially prepared reagents, although similar characteristics have been observed during the course of monoclonal antibody workshops.

**The acquired B problem**

Significant increases in the number of acquired B samples, referred to the immunohematology consultation service of the Community Blood Center of Greater Kansas City, were recorded during 1991-1992. It was soon clear that the increased number of acquired B samples was directly related to the use of newly introduced anti-B reagents based on clone ES4. This clone was known to cross-react strongly with galactosamine. Many examples of acquired B, some of them otherwise undetectable, were disclosed. Especially troublesome was the strength of ES4 reactions that, unlike those expected with polyclonal anti-B, were often as strong as that given with normal B cells. An interesting feature was the great variability of activity seen when the same RBCs were tested with the different ES4 reagents. The three manufacturers utilizing ES4 (Gamma Biologicals, Houston, TX; Immucor, Inc.; and Organon Teknika, Inc., Durham, NC), did so with formulations that differed significantly in terms of pH. Under acidic conditions, galactosamine (GalNH₂) becomes protonated to GalNH₃⁺, a structure that does not cross-react with anti-B. The pH of final reagents therefore is critical. This is consistent with the observations of Beck’s group that cells expressing the acquired B phenotype were most strongly agglutinated by Gamma-clone anti-B (pH 7.14), less strongly by Immucor anti-B (pH 6.9) and weakest, often negative, by Organon-Teknika anti-B (pH 5.9). The consequent blood grouping confusion associated with the sensitivity of ES4-based reagents for acquired B probably resulted in a fatality and led to a requirement that these reagents be reformulated at acidic pH. Reformulation seems to have had the intended effect. The examples of acquired B recognized with current ES4-based reagents are likely those that would have been detectable by human polyclonal anti-B.

Reformulation was not without its own problems, however. Two anecdotal accounts of ABO blood grouping anomalies were reported to the 47th annual meeting of the American Association of Blood Banks (1994). Both cases were associated with the testing of plasma or serum suspended RBCs. Agglutination, ostensibly attributed to anti-B, was ultimately related to activation of pH-sensitive cold agglutinins by the acidic environment provided by the ES4 reagent. Kirkegaard et al. confirmed these findings and demonstrated the potential incidence of these cold agglutinins to be in the order of 1 in 500 donors and the specificity to be toward Prα.

**Anomalies Related to Reagent Manipulation by the User**

Another blood grouping discrepancy familiar to most blood processing centers occurs when different reagents are used to test the same sample. Variations on this theme include the blood center’s failure to detect weak subgroups of A with their automated equipment utilizing diluted reagents, which are detected by the hospital using the same reagents undiluted in a test tube. We have recently encountered some extreme examples of this situation in Kansas City. The first case concerned an 18-year-old female donor considered by our automated blood grouping instrument to be group O. However, the hospital detected a very weak reaction with Immucor’s anti-AB monoclonal reagent. Further studies at the blood center confirmed the positive reaction with the anti-AB reagent but only in a test tube. The positive reaction was attributed to anti-AB clone ES15. Family studies revealed other members with even weaker A expression detectable only by adsorption and elution using anti-AB ES15. These super-weak reactions were confirmed by DNA analyses.

Another example occurred in the routine blood typing of an autologous male donor. We considered this donor to be group O although no anti-A could be detected in his serum. Direct agglutination tests were always negative but the results of adsorption and elution tests with ES15 were suspicious but not convincing to all members of staff! Again, DNA studies demonstrated this individual’s genotype to be O/A1.

On other occasions, discrepancies are brought to light only through the use of different clones of the same alleged specificity. For instance, another young female (first-time donor) was thought to be group O by our blood center. The hospital, on retyping, was convinced that she was group B! The hospital was using a particular monoclonal anti-B. Further studies at the blood center confirmed the hospital’s finding but only with that
particular monoclonal anti-B. Another reactive monoclonal anti-B was found among a panel submitted for evaluation by the 1996 ISBT Monoclonal Workshop. This case may represent an extreme example of the dependence of serologic activity on fastidious specificity. At the time of writing, family studies had not been performed.

The dilemma posed by monoclonal reagents (with the exception of the last case) as the cause of the anomalies discussed above is rooted in their high potency. Clearly, the B(A) and A(B) phenotypes as well as the previously unsuspected incidence of acquired B and weak subgroups are not new phenomena. Their discovery awaited the use of sufficiently potent reagents. The availability of potent monoclonal antibodies has provided a source of routine blood grouping reagents without which safe transfusion could not proceed. Their use has also moved ABO blood grouping to a new level of sensitivity, which is clearly approaching unnecessary extremes as evidenced by the frequency with which discrepancies are encountered between current and historical results. These reagents will occasionally challenge users to provide explanations for an apparent change of blood group to medical professionals, as well as to patients and donors—the latter groups may well prove to be more problematic. Accrediting agencies should not regard these instances as errors but rather as limitations of previous reagents or as novel characteristics of current reagents.

New Attitudes Toward Anti-D Reagents

Traditionally, routine D typing has not been considered a matter for much conjecture. Manufacturers provided perfectly suitable reagents formulated from human polyclonal anti-D. However, the availability of Rh immune globulin for hemolytic disease of the newborn (HDN) prophylaxis and a change in attitude toward deliberate stimulation of volunteers has disrupted the steady supply of human antibodies on which this system depended. Monoclonals have again come to the rescue.

The first monoclonal anti-D was reported by Crawford and colleagues in 1983. Early attempts by the makers of ABO monoclonals to produce a monoclonal anti-D was complicated by technical problems. Solutions to cell line stability problems came in the form of human-mouse heterodomas. Several potent monoclonal anti-D reagents have now been produced. Predictably, the finely focused nature of monoclonal anti-D reagents in an antibody that lacks the broad specificity of polyclonal anti-D. It is unlikely that even complex blends of monoclonals can entirely reproduce the serologic repertoire of polyclonal anti-D. It has been questioned whether a single monoclonal reagent can be formulated to serve the typing requirements of both donors and patients. Current formulations attempt to broaden the specificity spectrum of monoclonal anti-D with polyclonal augmentation. Clearly, this approach remains inherently dependent on a continued supply of polyclonal anti-D.

The specificity problems characteristic of monoclonal anti-D concern the detection of weak and partial D phenotypes. Many IgM monoclonal anti-D reagents have been developed that react well with weak D phenotypes by direct agglutination. Unfortunately, these reagents fail to detect some partial D phenotypes. D*V is a case in point. Other monoclonal anti-D have been made that react well with category D*V cells but usually do not give acceptable reactions with other weak D phenotypes. The significance of category D*V depends on the clinical setting. The detection of D*V in donors is controversial. Some recommend that because the immunogenicity of D*V is largely unknown, donor blood should be tested with a reagent that will detect this D variant. This approach could require an additional test as suggested by Jones and colleagues. A more cavalier argument deems the detection of D*V as unnecessary: a hypothetical viewpoint relying for its plausibility on the paucity of data incriminating D*V as immunogenic. Proponents marshal the following issues to their cause: (1) There is a relatively low D-site density on D*V cells; (2) the epitope display on D*V is extremely restricted; (3) the D epitopes believed to be important to immunogenicity (eps 6/7) are absent from D*V cells; (4) the frequency of D*V is in the order of 1 in 5000; and most significantly, (5) there are no published data that support the view that D*V is immunogenic. Therefore, there is no perceived need to require that anti-D used for donor typing be capable of detecting D*V. The possible flaw in this argument is to attribute the lack of published accounts of immunization to the five points raised above. In fact, as Issitt sagely points out, the absence of evidence of immunization may simply be that past practice prevented its occurrence. In other words, until comparatively recently, the standard anti-D typing reagent was a polyclonal anti-D that detected category D*V cells and classified them as Rh-positive, thereby denying any immunogenic opportunity by transfusion to D- recipients. Whether or not sensitization occurred through pregnancy is unknown because the observation of maternal anti-D would lead to the finding of the expected D+ status of the infant.
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with no attempt made to distinguish between a frankly D+ status and a partial-D status. To quote Issitt, "Thus it is entirely possible that partial-D red cells of category D\textsuperscript{V1} have stimulated production of anti-D in D-negative persons but that the events have gone unrecognized because there was no reasons to suspect them."\textsuperscript{15} In light of this reasoning, Issitt cautions, "it would seem to be a highly dangerous exercise deliberately to type category D\textsuperscript{V1} donors as D- without much better evidence that such red cells will not stimulate productions of anti-D." Thus, Issitt agrees with the position of Jones et al.\textsuperscript{14} who advocate that donors should be typed with a reagent that will detect partial D\textsuperscript{V1} to protect against the theoretical risk of immunization by D\textsuperscript{V1}. (This author continues to be baffled that mighty intellects contentiously debate strategies to avoid these theoretical risks while accepting the far greater daily threat of immunization to K, Fy\textsubscript{a}, Jk\textsubscript{a}, etc., as inherent to transfusion therapy.)

The requirements for a typing reagent for patient use may be less controversial. Jones and colleagues conclude that it is safest to regard category D\textsuperscript{V1} patients as D- to ensure that they receive D- blood because D\textsuperscript{V1} recipients of D+ blood readily make anti-D capable of causing HDN.\textsuperscript{14} This strategy also ensures that D\textsuperscript{V1} mothers receive prophylactic anti-D (Rh immune globulin). Thus, it is recommended that monoclonal anti-D used for typing transfusion recipients or used for prenatal testing should not detect D\textsuperscript{V1}. Adoption of the policies advanced by Jones and co-workers would require the routine use of two sorts of anti-D reagents: one capable of detecting D\textsuperscript{V1} to be used for typing donors, the other, which will not react with D\textsuperscript{V1}, to be used for typing of patients. In the absence of a reagent that more closely mimics the serologic activity of polyclonal anti-D, the use of specific reagents for donors and patients may have to be given serious consideration. A further complication exists regarding the D variant R\textsubscript{0} Har. Because the majority of IgM monoclonal anti-D react with this variant, R\textsubscript{0} Har individuals will be considered D+, transfused with D+ blood, and, therefore, R\textsubscript{0} Har mothers will be denied the protective benefits of Rh immune globulin. Immunization of an R\textsubscript{0} Har mother through the blood of her D+ infant has been recorded.\textsuperscript{16} This did not occur when D typing was routinely performed with polyclonal anti-D.

Fortunately, the incidence of R\textsubscript{0} Har appears to be low. However, this might prove to be of little comfort to an immunized mother. Clearly, the ideal monoclonal anti-D has not yet been designed to offer all the characteristics of the traditional polyclonal reagent.

Conclusions

This report represents an attempt to reproduce my seminar presentation, which was given in my usual informal style. I hope I have included most of what I said, at least anything of scientific interest. My intent was not to catalog a series of problems encountered with monoclonal reagents but rather to emphasize some rare but important characteristics unlike those associated with polyclonal reagents. Although these anomalies may be considered rare, blood centers that process 100,000 units or more are almost certainly bound to make their acquaintance, probably several times each year.

The most important part of my presentation, however, was to wish my dear friend Peter Issitt a long, healthy, and happy retirement.

References

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