Today, when monoclonal antibodies (mAbs) have become one of the most important classes of therapeutic drugs, it is easy to forget how much they have transformed our healthcare in other ways. One of the first clinical areas, as this paper shows, where mAbs made their mark was in the field of blood typing. The adoption of mAbs for this purpose was done with little public fanfare or funding. Nonetheless, it radically transformed the accuracy and cost of blood typing and shifted the procedure away from a dependence on reagents made from human blood donated by volunteers. This paper argues that the development of mAbs as reagents for blood typing laid the foundation for the first large-scale production of mAbs thereby paving the way to the advent of mAb diagnostics and therapeutics.

Introduction

Since the mid-1990s monoclonal antibodies (mAbs) have become one of the most important classes of medicines. Today they comprise 6 out of the 10 best-selling therapeutic drugs in the world and constitute a third of all new drugs introduced. Such drugs are radically transforming the landscape for the treatment of cancer and autoimmune disorders. Yet, the recent success of mAb therapeutics is only a small part of the ways in which mAbs have changed healthcare. One of the first clinical areas where mAbs made their mark, which is frequently forgotten, was in the field of blood typing, a procedure routinely carried out to ensure safe blood transfusions and blood banking. How mAbs came to play a pivotal role in this field is the subject of this paper and is illustrative of how the technology helped transform healthcare in general. Drawing on oral interviews, cited here as personal communications (PC) with key scientists* and the examination of their unpublished papers alongside published literature, this paper traces the twists and turns in the development of mAbs for blood typing. What is noticeable about this history is that the use of mAbs for blood grouping did not attract any major public funding or fanfare. Instead, they slipped quietly unobserved into routine clinical practice. This paper shows that while the development of mAbs for blood typing remained largely hidden from public view, their emergence helped shift the procedure away from a dependence on reagents made from human blood donated by volunteers and radically transformed the accuracy and cost of blood typing. The development of mAbs as reagents for blood typing also established the basis for the first large-scale manufacture of mAbs thereby laying the foundation for their use as diagnostics and therapeutics.

Keywords: blood typing, manufacturing, monoclonal, production

Abbreviations: LMB, Laboratory of Molecular Biology; FDA, Food and Drug Administration; MRC, (British) Medical Research Council; mAb, monoclonal; NHS, (British) National Health Service; PC, Personal communication; RH, Rhesus

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however, was not adopted overnight. Indeed, many scientists failed to grasp its significance at first. How little importance was attached to the technique is revealed by the case of the British National Research Development Corporation, a governmental body responsible for patenting innovations arising from UK Research Councils funding, which decided not to patent it. Their rationale was that they could not envision it leading to any commercial products.

The rise of the serological test for blood grouping

The procedure for blood grouping long pre-dated the development of monoclonal antibodies. Physicians had been carrying out blood transfusions since the 17th century. This practice remained dangerous for many years, often resulting in the death of patients. Its safety, however, greatly improved as a result of the work of the Austrian immunologist Karl Landsteiner in the early twentieth century. Mixing blood from 2 different individuals, Landsteiner discovered that humans do not all share the same type of blood. From this, he determined on the basis that red cells (erythrocytes) which manifested agglutination before proceeding with transfusion.1,2

Initially, the adoption of serological testing of blood before transfusion was limited. By the 1940s, however, it had become a common procedure conducted ahead of transfusion for working out ABO incompatibilities. In 1945, further improvements were made as a result of the work of Robin Coombs, an English pathologist at Cambridge University researching hemolytic disease among newborns, a condition brought about by Rh incompatibility which results in antibodies from a pregnant mother’s blood destroying the blood of her baby in her womb. Importantly, he devised a test for identifying antibodies reactive with erythrocytes which fell outside of the main ABO blood grouping system. His test was critical given that other blood groups had now been identified in addition to Landsteiner’s 4 and the fact that severe transfusion reactions were still common. The Coombs’ test not only helped in the management of rhesus incompatibility, but reduced the problems associated with transfusion.1,2

By the late 1970s, 2 types of tests existed for blood grouping. Both of these tests had the advantage that their results could be detected with the naked eye. Quick and routinely used in emergency medicine, the first test involved mixing the blood sample to be typed with antiserum on a blood grouping glass tile and checking for any signs of agglutination. The second test required mixing the blood sample with antiserum in a test tube with a saline solution and letting the test stand for 2 hours before checking for the presence of any sedimentation which was a sign of agglutination. This process could be expedited using a centrifuge in emergencies. Of the 2 tests, the second was the most common one performed in hospitals.1,3

Milstein quickly grasped the viability of using mAbs for blood typing. All blood grouping tests at this time depended on conventional polyclonal antibodies drawn from antiserum. These antibodies acted as markers that would bind and clump specific determinants of the red blood cells. It was this that helped determine the blood group classification. Up to the late 1970s the bulk of antiserum used in blood typing was sourced from humans. In Britain this was secured from screening blood donated by volunteers to the National Health Service (NHS). Elsewhere, such as in Europe and America, most antiserum was supplied commercially, being secured from blood drawn from hyperimmunised donors, that is people specifically immunised with samples of purified blood group substances different to their own. Such immunisation, however, was potentially hazardous, as volunteers could either develop reactions to the procedure or risked infection from diseases like hepatitis that were sometimes contaminated the plasma used for immunisation. While risky for volunteers, hyperimmunised donors provided a higher potency of antisera than that obtained from screening the general pool of blood donated to the NHS, being of a sufficient potency to meet the very high standards required by the American Food and Drugs Administration (FDA). Hyperimmunised serum was also considered to be the most reliable reagent for use in the rapid tests done on glass tiles. By contrast, NHS antiserum reacted more slowly than hyperimmune based reagents so was unsuitable for emergency typing of certain groups of blood. The NHS reagents, however, were sufficiently reliable for use in the test tube-based method.4,5

Some efforts had been made to improve the potency of the NHS antibody reagents by the immunisation of some donors. Such hyperimmune sera, however, was expensive, reflecting the high degree of labor and time involved in its production. Firstly, volunteers needed to be immunised which was time-consuming. Following this, all samples taken from immunised volunteers required extensive assaying before they could be used, a process that had to be repeated for each sample obtained. The high degree of labor involved was reflected in the cost of such reagents, which in Britain in the 1970s were priced at between £250 and £600 per liter.5

Even the cost of antiserum obtained through the screening of blood donated to the NHS was high, costing on average £250 per liter. This, in part, reflected the large operation involved. Some idea of the
scale of the work can be seen from the fact that the nation’s Blood Group Reference Laboratory required 1200 liters of human serum generated from 6,000 blood donations every year to carry out blood typing. The system for collecting such blood was also considerably wasteful. At least 30 per cent of all sera collected from the large number of small individual donations which underwent detailed screening was discarded on account of failing to meet the required standards. An additional problem with the screening method was the fact that only 7 per cent of the British population fell into the blood group B category, which was an important source for anti-A serum. This meant that supplies of potent anti-A serum were limited. As a result hospitals had to buy such serum from commercial outlets. Obtained from hyperimmune donors this serum could cost £450 per liter.4

Over the years other sources were evaluated for securing antibodies for blood typing. This included ones drawn from trouts’ eggs and snails. Such efforts, however, came to nothing. Overall, the supply of appropriate antiserum was limited. Furthermore, what little was available was under severe strain as a result of an upsurge in blood typing to meet the increasing frequency of major surgery and the fact that human blood serum was critical also for other medical purposes. The shortage was not helped by the fact that no 2 human sera are alike and sufficient volumes of antiserum are difficult to secure with adequate antibody potency for generating reliable results.  

mAbs—a potential reagent for blood grouping

Milstein quickly grasped the potential mAbs held for improving reagents for blood typing. Not only could they offer potent reagents amendable to standardization, their supply was perpetual. As early as 1975, he attempted, with Köhler, to produce mAbs against the Rhesus (RH) blood group. The 2 scientists, however, made little progress. More success was to be had in generating a mAb against another group of blood cells known as type A. This emerged by chance out of the work Milstein did with Alan Williams, an Australian immunologist based in Oxford, to develop mAbs for differentiating antigens on the surface of immune cells found in rats. (PC, Milstein).8 Based on this, Milstein launched a collaboration to develop mAbs for blood typing with Douglas Voak, a pathologist based in the Regional Transfusion and Immuno-Hematology Center, Addenbrooke’s Hospital, which was just over the road from his laboratory. While the first mAb reagent they created had some problems, within a short time they had to hand one which experimentally proved an effective tool for typing blood group A.(PC, Milstein) Soon after, the 2 scientists were joined by Steven Sacks, a medical graduate who had joined the LMB in 1978 to work as a doctoral student under Edwin Lennox, an American immunologist and founding member of the Salk Institute who had come to Cambridge in 1974. Sacks was curious to understand a strange phenomenon he had observed among some mAbs generated against different cancer tumors. These had been made as a tool to investigate the factors involved in the stimulation of immune responses to tumors. When placed on a small plastic plate together with a mixture of cancer cells, the cells displayed an unusual clumping in a disc-like formation. Surprisingly, he found that in addition to binding to tumor cells, the mAbs appeared to target other cells. With the help of Voak and Milstein, Sacks discovered that he had inadvertently identified a mAb that bound powerfully to antigens found on the surface of human blood group type A.(PC, Sacks) Further investigation revealed this had come about because they had immunised a mouse with human bowel cancer cells originating from a patient with the blood group type A. The mAb they had created only seemed to target group A red blood cells and not group B or O cells. They subsequently found out that mice most commonly produce anti-A antibodies.4 (PC Milstein)

Initial test results from Sacks and Lennox’s anti-A mAb disappointingly indicated it to be a weak reagent for blood typing compared with human serum. This dulled the team’s enthusiasm for about a month, but their excitement was rekindled when they developed a second anti-A mAb, purposefully this time. Tests indicated it was potentially a potent blood typing reagent. Critically, the mAb showed several advantages over conventional typing sera. Describing the results, Sacks wrote, “Monoclonal anti-A produces a clearly visible reaction with red cells, improving the recognition of A and AB cells. The clearest benefit is seen with the weaker blood types of A poorly detected by conventional grouping serum… Our present reagent is about 3 times as potent as conventional serum. Additional improvement in the speed and strength of red cell clumping can be achieved by concentrating the monoclonal antibody 4 times so that it equals the potency of hyperimmune commercial serum.”

Sacks reported the mAb was particularly well suited for use in a blood grouping machine. The first automated blood grouping machine had appeared in 1963. This automatically pipetted samples and reagents into sample wells. By the mid-1970s the automation of blood typing had been advanced through the introduction of plastic microlitre plates, solid phase and gel technologies. The advantage of these new technologies was they decreased the volume of reagent required. Nonetheless, the process continued to require a considerable amount of work on the part of the technicians, who still had to manually prepare reagents, identify a patient sample and interpret and record the results. By the time the Cambridge team at Cambridge began their work, automation had begun to improve as a result of the introduction of laser scanners and computers which made it possible to identify and record labeled samples. The process involved mixing red cells with an antibody in one of several channels in the machine which could be scored for agglutination by passing through a light beam or inspecting ejected samples of the cells on blotting papers. It was easy to see how useful mAbs could be to the automation of blood typing. Sacks reported that machine operatives greatly favored their anti-A mAb reagent because, as he put it, “the tighter pattern of red cell clumping enabled detection of weak A.” Following the successful creation of the anti-A reagent, Sacks turned his efforts to developing an anti-B mAb. This proved more difficult than anticipated. Part of the
problem was that most of the mice they immunised produced anti-A mAbs. Eventually Sacks created an anti-B mAb after immunising many different strains of mice with a group B blood group substance.(PC, Sacks)\textsuperscript{10}

By 1982 the team had developed a number of anti-A and anti-B mAbs and investigated their use as blood typing reagents for more than 3 years. Out of this they had identified one anti-A and one anti-B mAb that were good for routine use in ABO blood typing. The 2 mAbs had been evaluated against thousands of blood samples done both manually and through machines. Overall, the anti-A mAb had been tested against 91,000 samples, and the anti-B mAb against 65,000 samples. The results indicated that the mAbs offered greater sensitivity than conventional antiserum for detecting different sub-types of each blood group and importantly did not give any false-positives.\textsuperscript{5}

The team believed the adoption of mAbs as reagents for blood typing offered a significant advantage in terms of reducing costs. The base line they used for the cost savings was hyperimmune serum, which on average cost £250 per liter. As they argued,

The application of monoclonals would provide reagents at an estimated cost of £150/liter, saving an estimated £100,000 on a yearly production of 1,000 liters. … The financial saving from using monoclonals includes the benefit of saving the 6,000 donations at present used annually to make NHS reagents. This is less than 1% of all the plasma used to prepare antibody reagents. This represented one of the largest production quantities. The supply of mAbs came from the growth of hybridomas, which would grow in the animals’ ascites, fluid that gathers in the abdomen. Ascites thus produced the greatest concentration of mAbs, but this method was unsuitable.\textsuperscript{5,9} Many within the company saw this as not only impractical, but also unethical.\textsuperscript{13,14}

The challenge of up-scaling mAb production

Celltech’s development of mAbs for blood typing was one of the first ventures into the large-scale applications of mAbs and as such posed several issues. One of the prime challenges facing Celltech was finding a means to improve the yield of mAbs in a way that was cost-effective or even less than the costs involved in the production of blood reagents then on the market.\textsuperscript{12-14}

Celltech’s foray into the commercialisation of mAb-based blood reagents catapulted it to the forefront of improving mAb manufacturing methods. With ascites discounted as an option for production, Celltech focused its efforts on optimising cell culture production. One of the advantages of cell culture was that it would be feasible to scale-up the size of the vessel in which the mAbs were produced and the engineering process was reproducible. In looking to develop its cell culture production of mAbs, Celltech decided to draw on previous industrial techniques developed for vaccines which had started in the 1950s for scaling-up the production of the polio vaccine. The polio vaccine had been the first commercial product generated using mammalian cell cultures, using monkey kidney cells as the starting basis. Further improvements had been made to the process though the commercial production of vaccines for rabies and foot and mouth disease in the 1960s.\textsuperscript{15-17}

One of the most important sources of expertise for Celltech came from Burroughs Wellcome, a major British pharmaceutical company. The company had developed a system, during the 1970s, for commercial production of vaccines for rabies and foot and mouth disease in the 1960s.\textsuperscript{15-17}

The main cost benefit of monoclonals stems from their being produced in large batches, which reduces the expensive testing workload by more than 90%. At present tests are on batches of 2.5 liter antiserum pools, requiring initial screening tests and 13 detailed studies on 12 individual serum donations. Tissue culture-produced mAbs reagents need only a few tests to monitor potency during culture and one detailed set of tests on the final batch of perhaps 100 or more liters of culture medium.\textsuperscript{5}

A significant advantage of the hybridoma technology was that it provided reagents that did not change over time. In this way everything that was learned about one batch could be reused for the next.\textsuperscript{10}

Commercialisation begins

Armed with these positive results and the backing of the MRC, Lennox and Sacks filed for patents in 1981. Granted in Europe, Japan and the USA, these patents provided the basis for producing mAbs as reagents for ABO blood testing. The company that led the commercialisation of the reagents was Celltech, a British biotechnology company formed in November 1980 with British government support. In 1983 Celltech estimated that the world market for such products was worth £7.5 million in terms of hospital sales and £3 million for other sales. To help it in its effort Celltech hired Lennox as its director of research and launched the testing of mAbs as blood reagents at 34 hospitals and transfusion centers in Britain.(PC, Milstein)\textsuperscript{11,12}

The development of mAbs as blood reagents necessitated Celltech entering completely uncharted territory. Up to this moment in time most applications investigated, such as a tool for research or diagnosis, or for the purification of a natural product like interferon, required only a small quantity of mAbs. By contrast blood grouping would require many kilograms of mAbs. The difficulty Celltech faced was that until now the 2 basic procedures for producing mAbs yielded only very small quantities. The supply of mAbs came either from the growth of hybridomas grown in cell culture in vessels or by the insertion of hybridomas into rats or mice which would grow in the animals’ ascites, fluid that gathers in the abdomen. Ascites produced the greatest concentration of mAbs, but this method was unsuitable. Given that 20 mice yielded one gram of purified mAb, Celltech would need 20,000 mice to produce 1 kg of mAb.
in cell culture production. Its production system laid the basis for production of many other protein based drugs, including those made through genetic engineering. (PC, Christie)

By the time that Celltech’s team began looking to improve the cell culture of mAbs, most mAbs were being generated on a small-scale, using either static flasks or roller bottles. To scale-up this process, Celltech scientists took advantage of the fact that hybridoma cells could be grown in suspension and began to develop a process that could be done in deep-tank fermenters. Such vessels were common in industry, being used for the growth of micro-organisms, such as bacteria for antibiotics. The company’s scientists chose to adapt an airlift fermenting reactor, first used by Burroughs Wellcome for producing human interferon. One of the benefits of this fermenter was that it provided the simplest means for monitoring and controlling the environment around the cells, thereby enabling a more predictable scaling-up process. Within a short time, Celltech had developed a means to produce mAbs on an industrial scale. This was achieved through the use of airlift fermenters in tandem with improvements made to the culture medium used for growing hybridomas. Such measures enabled Celltech to produce mAbs in 1,000 liter fermenters. While the productivity of each hybridoma cell line varied greatly within these fermenters, by 1986 Celltech had achieved a yield that represented a 4 to fivefold increase over those obtained in simple laboratory culture systems such as roller bottles.” In 1989 Celltech’s chief executive officer, Fairtough reported his company had 2,000 liter and one 2000 liter airlift fermenters in operation as well as many smaller-sized vessels. Overall the company had produced 100 different cell lines in quantities over 100 grams, totalling 6 kilograms of mAbs. This amount put the company ahead of many of its competitors.

mAbs slip into routine blood grouping practice

Some idea of Celltech’s achievement can be seen from the fact that by 1989 over half of the world’s blood-typing reagents were based on mAbs produced by the company. Celltech’s success reflected a wider uptake of mAbs as blood reagents, which grew considerably in the 1980s. Their popularity was rooted in the fact that their quality often exceeded that of conventional US FDA licensed reagents. Moreover the technology afforded uniform batch-to-batch control, so more readily fulfilled the FDA licensing requirements than conventional reagents. The mAb reagents were also free of the contaminants which was not the case with conventional blood serum. On top of this, large-scale production was now feasible and more effective than securing antiserum from human volunteers. Now 1,000 liters of mAb supernatant could be produced in just 18 days, the equivalent of 2,000 individual human donations gathered through an automatic collection process. Importantly, the mAb reagents helped eliminate the workload involved in evaluating large numbers of small individual donations. The successful creation of mAb reagents for blood typing also ethically challenged the continuation of the risky practice of immunising volunteers to secure conventional reagents.

The demand for mAb based ABO blood reagents was reinforced by the spread of the human immunodeficiency virus (HIV) and concerns about the safety of blood banking and transfusion. Nowhere was this more apparent than in France, where a public scandal emerged during the mid-1980s as a result of the discovery that hemophiliacs and transfusion recipients had been given blood infected with HIV. In France the uptake of mAbs as substitutes for human plasma-derived products was initially slow, but increased rapidly in the late 1980s. Between 1985 and 1987 the use of mAbs as reagents for blood typing of ABO blood groups before transfusion grew from 18.8 per cent to 35.7 per cent. Thereafter the percentages grew to 48.7 per cent in 1989, 56 per cent in 1993 and 82.2 per cent in 1995. Despite the rapid success of mAbs for ABO blood typing, the ability to create mAbs for other blood groups took longer to materialise. This was particularly noticeable in the case of anti-Rh mAbs which Köhler and Milstein had failed to prepare in 1975. Such a mAb was highly sought after, with the Rh blood group being one of the most clinically important group after ABO. It was particularly critical because plasma that was rich in anti-Rh antibodies had become increasingly scarce by the 1970s. In part this reflected the fact that the number of women naturally sensitized during pregnancy had declined as a result of better management of hemolytic disease. Mothers known to be rhesus negative bearing infants with rhesus positive blood had, since the early 1970s, begun routinely to be injected during their pregnancies with antibodies collected from the plasma taken from immunised volunteers. This treatment had proven highly effective at preventing hemolytic disease among newborns as well as complications among mothers. Ironically the success of the treatment depleted stocks of the plasma while at the same time increasing demands for it. Alternative sources of the plasma could be secured from donors following a mismatched blood transfusion or by the deliberate immunization of volunteers. Immunisation, however, was not satisfactory because it required donors having booster injections of red blood cells to secure plasma with adequate potency of antibodies, which in itself carried risks.

One of the problems scientists faced was the fact that mice do not make antibodies to Rh blood antigens. In 1983 some headway was made by researchers based at the University College London and the North East Thames Regional Blood Transfusion Center by using the Epstein-Barr virus to modify B lymphocytes taken from donors with hyperimmune anti-D antibodies. These first few mAbs, however, proved less effective than conventional human antiserum. In 1986 a final breakthrough was achieved as a result of a collaboration between scientists in Cambridge and Oxford. This provided reagents for both rapid emergency slide tests as well as for the saline based test tube technique. Despite their utility, initial uptake of the reagents varied globally. France, for example, showed faster adoption than the United States because of the country’s difficulty in obtaining plasma that was rich with anti-Rh antibodies.
In tandem with the development of mAbs for typing the Rh blood group, mAbs also began to be produced against many other blood groups. By 1989, for example, mAb based reagents had virtually displaced all use of conventional serum for typing M and N blood groups. In addition to offering a new source of reagents, the adoption of mAbs for blood typing obliged producers to improve the quality and standardisation procedures they had in place for the production of blood reagents overall. Furthermore, the mAbs provided powerful tools for extending knowledge about the structure of antigens found on the surface of red blood cells which helped advance improvements to blood typing tests.18,19

Conclusion

The adoption of mAbs as typing reagents for blood grouping was just the start of an avalanche in the application of mAbs for improving healthcare. By harnessing the power of mAbs for blood typing, scientists working within the confines of an academic laboratory helped establish both the clinical utility of the new technology and its commercial potential. In so doing they forged an important bridge between the academic world and that of industry. These efforts helped pave the way to scaling up the production of mAbs. No longer was production dependent on the skills of a few university-based researchers who could provide only limited quantities. This emergence of the new industrial manufacturing infrastructure would prove fundamental to the development of mAbs for much wider applications, including diagnostics and therapeutics.

* The interviews cited in this paper are:
  César Milstein, interviewed by David Secher, no date, transcript, Laboratory of Molecular Biology.
  Steven Sacks, interviewed by Lara Marks, 11 Nov 2011, notes.

James Christie interviewed by Lara Marks, 12 Nov 2012, notes.

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