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ESSENTIALS The development of rodent monoclonal antibodies opened the door to the creation of antibodies specific to soluble and cell-surface antigens. ‘Humanized’ therapeutic antibodies have emerged as blockbuster drugs for the treatment of cancer, immune, and inflam- matory disorders—the so-called biologics. In this short chapter, two scientists who made seminal contributions to this field and remain actively engaged in its development give a personal account of how these remarkable developments came about. Introduction There has been a revolution in the pharmaceutical industry: anti- bodies have emerged as major blockbuster drugs for treatment of cancer and immune or inflammatory disorders. Much of this revo- lution was spearheaded in Cambridge, England, initiated by the re- search of Cesar Milstein and George Köhler at the MRC Laboratory of Molecular Biology and who, with N.K. Jerne, shared the 1984 Nobel Prize for Medicine or Physiology. As related in this personal perspective, Cambridge scientists and clinicians took up the chal- lenge to develop the original murine antibodies into powerful phar- maceuticals that can be administered repeatedly without the dire consequences of alloimmunization.

Monoclonal antibodies The technological discoveries related to the generation of rodent monoclonal antibodies (mAbs) by Köhler and Milstein in 1975 opened the door to the creation of antibodies specific to soluble antigens and to cell-surface antigens. Such antibodies not only had the potential to kill the cells or block the molecules involved in dis- ease processes, but were amenable to industrial production in cell cultures. There were, however, several uncertainties about their po- tential as therapeutic agents. For example, it was not clear whether (as agents directed to a single site on a cell-surface antigen) they would be capable of recruiting lytic payloads of the body’s comple- ment system and myeloid cells. Nor was it clear whether the im- munogenicity of rodent mAbs antibodies in humans would lead to human antimouse antibodies that would block therapy. Indeed, by the mid-1980s, immunogenicity was emerging as a key concern for the application of mAbs as therapeutic agents. We were witnesses to the discovery and early development of mAbs, and independently, and for differing reasons, embarked on research programmes leading to the reduction of the immunogen- icity of antibodies while ensuring therapeutic efficacy. One of us (HW) sought to reprogram the immune system to make it more tol- erant to foreign antigens, and to restore tolerance in autoimmune disease; the other (GW) sought

to use genetic engineering to render rodent antibodies as human as possible. Making ‘humanized’ monoclonal antibodies The starting point came from the work of several scientists, including the late Michael Neuberger, a close colleague at the MRC Laboratory of Molecular Biology. By genetic engineering Neuberger created mouse-human chimeric antibodies in which the antigen-binding (variable) domains of rodent mAbs were linked to the effector (con- stant) domains of human antibodies. Chimeric IgG antibodies, how- ever, comprised light chains that were only 50% human, and heavy chains that were 25% human, still leaving a substantial degree of ‘foreignness’ and potential for immunogenicity. GW reasoned that it should be possible to reduce ‘foreignness’ still further. It had long been supposed that the six hypervariable regions of antibodies, which were mainly loops located on one face of the asso- ciated variable domains, were responsible for binding antigen (and leading to their naming by Kabat as complementarity determining regions or CDRs). GW’s innovation was to replace the CDRs of model human antibodies by those from rodent mAbs, and thereby endow the human antibodies with the binding activities of the ro- dent mAbs. These ‘humanized’ antibodies could comprise as little as 5% foreign sequences, and as the CDRs differed between human antibodies anyway, it was suspected that they might be no more im- munogenic than fully human antibodies. Indeed, these antibodies were originally termed ‘reshaped’ human antibodies, and can, in this context, be regarded as a synthetic species of human antibody. 3.8 The evolution of therapeutic antibodies Herman Waldmann and Greg Winter

3.8 The evolution of therapeutic antibodies 297 Moving humanized monoclonal antibodies into clinical practice At the time of GW’s discovery, HW’s group in Cambridge had gener- ated a lytic rat antilymphocyte antibody (CAMPATH-1) with poten- tial in reprogramming the immune system in autoimmune diseases and transplantation, as well as for treatment of lymphocyte malig- nancies. Concerns about its potential immunogenicity were creating uncertainties for its development as a therapeutic agent, especially for repeat treatments. HW and GW decided to collaborate on the creation of a humanized version of the CAMPATH-1 antibody, but the work did not prove to be straightforward. We discovered that simply ‘transplanting’ murine CDRs onto a human framework was not sufficient to transfer antigen binding. This could, however, be ‘corrected’ by mutating framework residues thought to be important for the folding of the CDRs. We also knew that different human IgG isotypes varied in their ability to activate complement and harness Fc-dependent ‘myeloid-based’ lytic mechanisms, and showed that the human IgG1 isotype was the most effective for these lytic functions. We moved quickly to test the efficacy of the humanized antibody in the clinic. We were fortunate, as Geoff Hale in HW’s group had established a manufacturing facility in Cambridge, the Therapeutic Antibody Centre (TAC), where we could manufacture clinical grade antibody. The humanized CAMPATH-1 antibody was used for treat- ment of three patients at Addenbrooke’s hospital, two with lymphocyte malignancies and one with an intractable severe vasculitis. We were all amazed at the spectacular effects of the antibody in these three patients. In the two patients with lymphocyte malignancies we saw a substantial reduction of tumour mass without significant side effects, and the pa- tient with autoimmune disease underwent a long-term remission from the short-term therapy in what had been an otherwise refractory dis- ease. These exciting outcomes provided the platform for the evolution of Alemtuzumab/Lemtrada as a treatment in chronic lymphocytic leu- kaemia, and later—through work with Alastair Compston and Alastair Coles at the Department of Clinical Neuroscience—for the treatment of relapsing remitting multiple sclerosis. Furthermore, it helped to val- idate the use of humanized antibodies in the clinic, and to catalyse the antibody engineering revolution from which so many valuable new drugs have emerged in the past 25 years. Development of other types

of monoclonal antibodies The interest in creating human antibodies by genetic engineering did not stop there. GW and colleagues developed approaches to de- rive human antibodies from large libraries of human antibody vari- able domains, without the need to immunize animals for which GW was awarded a Nobel Prize in 2018. In turn this led to the develop- ment of Humira by Cambridge Antibody Technology in a collabor- ation with the biotechnology company Knoll, and this was the first fully human antibody to be approved for therapy by the US FDA. In parallel Marianne Bruggemann (at AFRC Babraham), Neuberger, and colleagues pioneered the development of transgenic mice with a human V-gene locus, allowing hybridoma technology to be used for the isolation of human antibodies from immunized mice. Other ingenious approaches have subsequently been developed to make human antibodies. The historical progression of engineering anti- bodies towards a more human form is outlined in Fig. 3.8.1, with many human-like antibodies emerging as effective therapeutics. The problem of immunogenicity Immunogenicity directed to CDRs does occur for many antibodies, including fully human antibodies, at least in some patients, al- though reporting of immunogenicity has not been as extensive as one might hope. As there is no natural tolerance to these CDR re- gions, additional strategies are desirable to eliminate that residual immunogenicity. One strategy has been to create mutations in the residual im- munogenic sites, such mutants being designed to eliminate the T-helper and/or B-cell epitopes of an antibody, but there is as yet no longer-term clinical evaluation as to what extent this is achievable. Another strategy has been to establish immunological tolerance to the immunogenic epitopes within the CDR regions. Building on classical studies on tolerance, HW noted that most foreign anti- bodies binding to blood cells (and thereby aggregated) were po- tently immunogenic, but that nonbinders (that did not aggregate) were able to induce immunological tolerance to themselves. Indeed, ‘FULLY’ HUMAN RODENT CHIMERIC HUMANIZED Fc VH VL CDRs (in blue) Fig. 3.8.1 The various engineered forms of therapeutic antibodies where the intention has been to replace rodent gene sequences with human derived ones. In blue are shown the regions of an antibody genetically derived from the rodent. In yellow are those derived from human genes. VH VL, variable domains of heavy and light chains, respectively; Fc, the fragment crystallizable region that carries antibody effector functions; CDRs, complementarity determining regions.

 by making a nonbinding mutant of the humanized Campath-1 anti- body, HW and colleagues were able to induce tolerance to the thera- peutic form. This approach may ultimately allow tolerogenicity to be built directly into therapeutic antibodies. Future prospects More generally the modular nature of antibodies has enabled a whole new generation of engineered antibody-based product. These have allowed variations in size and pharmacokinetics, and provided opportunities for incorporation of multiple specificities in the same antibody molecule as well as the addition of domains delivering a range of desired payloads. One burgeoning area where these novel constructs have been exploited is in cancer immunotherapy, where the intent has been to recruit and activate T-cells to tumours so as to exploit their lytic and diverse proinflammatory properties. In particular, encouraging outcomes have been seen from the use of bispecific antibodies, and of chimeric antigen receptors where anti- body variable regions have been connected to with T-cell receptor signalling domains. Continuing innovations based on understanding antibody mol- ecules and their functional interactions will surely generate new waves of therapeutic advances, targeting extracellular structures in ways that conventional small drugs have not yet achieved. FURTHER READING Bruggemann M, Neuberger MS (1996). Strategies for expressing human antibody repertoires in transgenic mice. Immunol Today, 17, 391–7. Coles AJ, et al. (2006). The window of therapeutic opportunity in multiple sclerosis: evidence from monoclonal antibody therapy. J Neurol, 253, 98–108. Gilliland LK, et al. (1999). Elimination of the

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