Review

Single B cell antibody technologies

Thomas Tiller[1]

Max Planck Institute for Infection Biology, D-10117 Berlin, Germany

Monoclonal antibodies (mAbs) are arguably the most significant class of biologics for use as pharmaceuticals and diagnostics. Many technological concepts exist for the generation and identification of therapeutically relevant mAbs, including the isolation and cloning of immunoglobulin (Ig) encoding genes from single B-lineage cells. This review summarizes various single B cell approaches and describes their use for the discovery of mAbs with potential therapeutic values or in basic research.

Contents

Introduction

Monoclonal antibodies (mAbs) are essential tools for many diagnostic applications and research investigations. In addition, mAbs have become ideal therapeutic reagents for many diseases and are well established in the treatment of cancer, autoimmune disorders and infectious diseases [1]. Many technologies exist for the generation of mAbs for both research and therapeutic purposes. Generally, for research purposes, murine mAbs are obtained by hybridoma technology that is based on the fusion of B cells, for example derived from immunized mice with myeloma cells [2]. In this way, mAbs against almost any antigen are created, but the direct therapeutic use of murine mAbs in humans is limited by both the high incidence of harmful immune responses against the murine, foreign protein and the murine antibodies lack of suffi-

cient effector function [3,4]. To alleviate these limitations, murine mAbs have been reengineered by chimerization and humanization technologies in which the murine constant and framework regions are replaced by human sequences [5–8].

Alternatively, human mAbs can be recovered from hybridoma B cell lines derived from immunoglobulin (Ig) transgenic mice with complete or partial human antibody repertoires [9–12]. One drawback is that the Ig transgenic mouse systems cannot precisely imitate a human immune response because of the effects of the murine genetic background on antigen processing and B cell regulation. As a result, the recovered antibodies might not display the precise specificities of naturally occurring antibodies in humans. Despite this drawback, the majority of the approved fully human antibodies today have been derived from Ig transgenic mouse systems.

To overcome the issues of murine systems, strategies for engineering mAbs created by an intact human immune system have been designed, which include the human hybridoma technology

E-mail address: thomas.tiller@morphosys.com.
[1] Current address: MorphoSys AG, D-82152 Martinsried, Germany.

Review

[13–15] and the immortalization of human B cells by transformation with Epstein-Barr virus (EBV) [16,17]. One major advantage of these methods is that they allow the isolation of native human mAbs with the preservation of the natural VH and VL pairing. These methods, however, are generally unsuitable for a comprehensive screening of large antibody repertoires, because of their highly selective and inefficient fusion and transformation events. In spite of this, improvements of these methods have yielded neutralizing mAbs, for example, against the H1N1 [18] and the H5N1 [19] influenza virus or the SARS corona virus [20].

Additional ways for creating and selecting mAbs include combinatorial display technologies, like phage, yeast and mammalian cell display [21]. Phage display has been successfully employed in the screening of large antibody repertoires and the isolation of mAbs against virtually any antigen of interest [22]. The combinatorial display technologies also allow for in vitro affinity maturation of selected antibodies [23,24]. One drawback, however, is that they usually rely on random combination and thus most likely unnatural VH and VL antibody pairings.

As it is thought to be important in antibody development to maintain the original VH and VL pairing as exists in human B cells, an efficient strategy has been designed which is based on the direct amplification of VH and VL region encoding genes from single human B cells and their subsequent expression in cell culture systems [25–32]. To facilitate rapid screening and detection of single cells secreting antibodies with the desired reactivity profiles from large populations of primary B cells cell-based microarray and microengraving techniques can be applied [33–37]. The single B cell antibody approaches harbor the potential to isolate functional mAbs reactive against conformational determinants that are present predominantly in vivo and difficult to emulate in vitro. The strategies straightforwardness, the requirement of relatively few cell numbers and the high efficiency in obtaining specific mAbs in a rapid way are balanced by the downsides, which are the need of adequate human donors and the limitation to certain target molecules. The following paragraphs describe the basic concept of single B cell antibody technologies and their use in basic research and in the selection of antigen-specific mAbs.

Concept of single B cell antibody technologies
Identification and isolation of single B cells
Depending on the research application, single B cell isolation can be performed either in a random way or in an antigen-selective manner from peripheral blood or from lymphoid tissues, for example the bone marrow. For random B cell isolation, procedures, such as, cell picking by micromanipulation [38], laser capture microdissection [39] and fluorescence-activated cell sorting (FACS) [30,32,40] have been described. Alternatively, selection of antigen-specific B cells has been demonstrated using antigen-coated magnetic beads [28], fluorochrome-labeled antigens via multi-parameter FACS [41–43], the hemolytic plaque assay [25] and a fluorescent foci method [44]. Furthermore, cell-based microarray chip systems [33,34] have been established for high-throughput screening of mAbs secreted by single antibody producing cells.

The major advantage of FACS technology in this application is that cells to be sorted can be clearly distinguished in terms of their stage of development and differentiation based upon the expression patterns of specific cell surface markers. In general, B cells at any stage can be sorted, but class-switched memory B cells and antibody-secreting cells (ASCs, i.e. plasmablasts and plasma cells) are of special interest to obtain relevant mAbs as they bear somatically mutated B cell antigen receptors (BCRs) with high affinities. For example, IgG+ memory B cells reactive to recombinant trimeric gp140 antigen have been successfully selected in patients with HIV by using fluorescently labeled gp140 [43,45]. By contrast, ASCs specific for a vaccine can be sorted without any antigenic labeling post immunization. For efficient recovery of specific mAbs, the donor's immune response must be assessed in this method, for example, by determining the frequency of ASCs in peripheral blood using an enzyme-linked immunospot technique (ELISPOT) before single cell isolation [30,46].

Additionally, high-throughput methods have been generated that lead to the efficient identification of B cells having the desired specificity before Ig gene cloning. One specific method is a soft lithographic method for microengraving [34,35]. Per screen up to one hundred thousand polyclonal B cells stimulated with a mitogen to develop into ASCs are deposited individually into an array of microscale wells on a chip. The array is then used to print a corresponding protein array, whereby each element contains the antibodies secreted by one single B cell. The antibodies on the microarray are screened with fluorescently labeled antigen(s) of interest, and mapped to the corresponding cells, which are then manually retrieved from their wells by micromanipulation and subsequently analyzed by clonal expansion or single cell reverse transcription-polymerase chain reaction (RT-PCR). A similar approach is the 'immunospot array assay on a chip' (ISAAC) method established by Muraguchis group [33]. As for the microengraving technology, single ASCs are placed on a microwell chip, but the chip's surface is coated with anti-Ig antibodies, so that the antibodies secreted by one individual ASC are captured on the surface around the well. The binding of biotinylated antigen to specific antibodies allows the detection with fluorescence-conjugates of streptavidin. Microengraving and ISAAC offer compelling advantages, such as the early and rapid identification of cells secreting antibodies with high affinities specific to an antigen of interest from a polyclonal mixture. Furthermore, these techniques simplify the screening of multiple antigens to allow the selection of different clones with distinct specificities.

Single cell Ig gene transcript amplification, sequencing and cloning
The construction of complementary DNA (cDNA) from single B cells provides an unbiased approach to the simultaneous analysis of expressed IgH and IgL chain genes [47,48]. Usually, single cell cDNA synthesis is performed in the original device that was used for cell deposition and cell lysis (e.g. the 96-well plate), which assures a convenient handling of large numbers of samples and minimizes the risk for cross-contamination. Notably, distinct B cell types show different amounts of specific Ig gene mRNA transcripts. For example, compared to memory B cells, ASCs contain relatively large amounts of Ig gene transcripts, thereby facilitating specific Ig gene amplification. Usually, full-length Ig gene transcripts are amplified by nested or semi-nested RT-PCR, whereby the RT might be performed as one-step reaction with the first round of PCR. Typically, forward primer mixes complementary to the corresponding IgH and IgL V gene leader sequences

and a single reverse primer specific to the constant region sequence are used [32,49–51]. If necessary, for example, when isotype independent cell sorting is performed, mixed reverse primers can be used for the amplification of IgH chains with different constant regions. In the second round, nested primer or primer mixes are used to increase sensitivity and specificity. Furthermore, restriction sites for subsequent cloning steps can be incorporated into the amplicons during this step. Alternatively, linear expression cassettes can be assembled that are directly transfected into mammalian cells for *in vitro* expression of the amplified Ig genes [52].

Strategies to link the rearranged IgH and IgL chain genes during amplification have also been explored [29,53]. For example, Meijer *et al.* developed a multiplex single cell RT-PCR approach that combines the amplified IgH and IgL chain products during the PCR through an overlap extension step. The joined DNA fragments are subsequently cloned into an appropriate expression vector for mAb production.

Although single cell RT-PCR methods are straightforward, not every protocol amplifies all functional IgH and IgL V genes because of limitations in the designed set of forward primers used. A method that allows the amplification of unknown 5′ ends of mRNAs is the '5′ rapid amplification of cDNA ends' (5′-RACE) technology. Although standard 5′ RACE techniques are unsuited for synthesizing cDNAs from single cells because of the usually small quantity of mRNA present, an improved protocol has demonstrated its suitability for the isolation of Ig genes from single B cells with promising efficiencies [54].

Regardless of the Ig gene amplification strategy used, the single cell Ig gene transcript information that encodes the specificity of the antibody is subsequently rescued by sequencing. The rearranged V, D and J gene segments can easily be identified and analyzed for the presence of mutations, insertions and deletions [55,56] using various databases, such as the IgBLAST search engine at the NCBI website (http://www.ncbi.nlm.nih.gov/igblast/).

Reactivity screening of antibodies

To determine the reactivity profile and biophysical characteristics of antibodies or fragments thereof, the proteins have to be expressed, purified and tested in various assays. Conveniently, in the cell-based microarray systems this reactivity screening can be performed before Ig gene cloning. To analyze the corresponding antibodies in more detail, however, molecular Ig gene cloning steps are indispensable, as large-scale amounts of protein are needed. The most common choices for expression systems are bacterial systems (e.g. *Escherichia coli*) or transient and/or stable mammalian cell systems (e.g. HEK 293, CHO cells). In *E. coli*, the cloned Ig genes are typically expressed as antigen-binding fragments (Fabs), whereas in mammalian cells expression can be in complete IgG format.

Single B cell antibody repertoire studies

Classic mouse and human antibody repertoire analyses on a cell-by-cell basis have significantly increased our knowledge about generic B cell immunology concepts. Initially, Ig genes from single B cells were studied on the genomic level by single cell PCR to elucidate the molecular basis of Ig gene rearrangement and allelic exclusion events [38,57–60]. As a next step, strategies were devel-

oped for the synthesis of cDNA from single B cells [48] to facilitate the unbiased description of mouse [47,61] and human [62] antibody repertoires. Substantial advances in understanding the expressed human antibody repertoire have then been made by the Nussenzweig group, which pioneered the investigation of defined B cell specificities during B cell development and differentiation [63]. The analysis of recombinant mAbs cloned from individual B cells of healthy individuals revealed that the majority of newly generated B cells in the bone marrow are self-reactive and that two checkpoints – one in the bone marrow and another in the periphery – select against self-reactivity during B cell development [40]. By characterizing alterations in the B cell repertoire of patients with deficiencies in innate immune pathways, the Meffre group suggested that such pathways are required for the removal of self-reactive immature B cells [64]. Based on the analysis of B cells from autoimmune patients it was proposed that a failure to establish tolerance at these checkpoints is associated with the development of Systemic Lupus Erythematosus (SLE) [65,66] and Rheumatoid Arthritis [67]. Further studies described a third checkpoint that selects against self-reactivity during IgM+ memory B cell differentiation [68]. Surprisingly though later in development, IgG+ memory B cells frequently express self-reactive antibodies in healthy donors [69,70]. In addition to these approaches which elucidate basic B cell biology, systems have evolved to isolate valuable mAbs from immune donors for potential therapeutic use or other applications, such as epitope discovery or vaccine design. Because of ethical concerns, humans cannot be exposed to any antigen at will. Therefore, it is clear that adequate donors have to be available from whom ASCs or memory B cells with antibodies reactive to an antigen of interest can be recovered. Particularly, vaccinated human subjects that can be followed up 6–14 days after immunization are ideal candidates from which to obtain such antibodies. As the immune system usually mounts a broad humoral response against an immunizing agent, mAbs reactive to multiple epitopes of the immunogen may be available. This was elegantly demonstrated by the Wilson lab, which engineered over 50 human antibodies specific to an influenza virus vaccine from ASCs isolated from vaccinated donors at day 7 after immunization [46]. Interestingly, as many as 80% of ASCs purified at this timepoint were influenza specific. The recovery of ASCs from peripheral blood at the peak of the immune response is essential, as these cells rapidly decline from the bloodstream as they migrate to the bone marrow to become long-lived plasma cells, which maintain humoral memory for a lifetime [71].

Alternatively, memory B cells can be isolated from the peripheral blood of naturally infected or vaccinated humans even months to years after antigen exposure. Although the exact correlation between antigen-specific memory B cell frequency and serum antibody titers has still to be clarified in humans, the determination of antigen-specific serum antibody titers is helpful in identifying appropriate donors. For example, Scheid *et al.* characterized the B cell memory response against HIV in six infected patients with broadly HIV neutralizing serum antibodies. In this study, 502 mAbs were isolated from single HIV-envelope binding memory B cells, 433 of them showing specific binding to recombinant gp140 antigen. Although none of the isolated mAbs was found to be broadly neutralizing across various strains of HIV, the combination of individual antibodies revealed a broad neutraliz-

ing effect [45]. A similar approach has produced neutralizing antibodies reactive to over 90% of circulating HIV-1 isolates [72]. Overall, these findings provide important insights into the immune response to HIV and are indispensable for rationale vaccine design strategies. Furthermore, single cell Ig gene cloning has revealed the kinetic and diversity of the antibody response against tetanus toxoid [73] and the Ig gene repertoire of anti-RhesusD-specific B cells [74]. In addition, recently, by using cell-based microarrays, mAbs against hepatitis B surface antigen have been prepared and characterized [75]. Overall, these studies demonstrate the versatility of single B cell antibody approaches in the production of valuable antigen-specific antibodies.

Summary and outlook

Over the last decade, single B cell antibody technologies have become an attractive approach to sample naïve and antigen-experienced antibody repertoires generated *in vivo*. The method has proved to be very useful in evaluating basic B cell biology concepts in health and autoimmunity. Furthermore, valuable mAbs,

have been isolated from vaccinated [46], naturally immunized donors [45] and patients with autoimmune disease [76]. In general, mAbs recovered by single B cell antibody technologies may be useful reagents for diagnostics, pharmacokinetic studies and clinical development. Major applications include the prevention of infectious diseases by passive immunization [77] and therapies for disorders currently medicated with serum IgG preparations [78]. A deeper understanding of neutralizing mAbs isolated from immune donors will also be helpful for rationale structure-based vaccine designs. Promising efforts in this reverse vaccinology approach are currently being made in HIV research, and should be applicable in other infectious diseases. In addition, future advancements on microfluidic platforms, including PCR cycling combined with next generation sequencing methods are likely to exceed the current systems and allow for high-throughput Ig gene sequence analyses of individual antibody repertoires. By increasing the availability of such repertoires, single B cell antibody technologies are likely to lead in the development of novel mAb therapeutics.

References

[1] Marasco, W.A. and Sui, J. (2007) The growth and potential of human antiviral monoclonal antibody therapeutics. *Nat. Biotechnol.* 25, 1421–1434

[2] Kohler, G. and Milstein, C. (1975) Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature* 256, 495–497

[3] Carter, P.J. (2006) Potent antibody therapeutics by design. *Nat. Rev. Immunol.* 6, 343–357

[4] Hwang, W.Y. and Foote, J. (2005) Immunogenicity of engineered antibodies. *Methods* 36, 3–10

[5] Almagro, J.C. and Fransson, J. (2008) Humanization of antibodies. *Front. Biosci.* 13, 1619–1633

[6] Boulianne, G.L. *et al.* (1984) Production of functional chimaeric mouse/human antibody. *Nature* 312, 643–646

[7] Jones, P.T. *et al.* (1986) Replacing the complementarity-determining regions in a human antibody with those from a mouse. *Nature* 321, 522–525

[8] Morrison, S.L. *et al.* (1984) Chimeric human antibody molecules: mouse antigen-binding domains with human constant region domains. *Proc. Natl. Acad. Sci. U.S.A.* 81, 6851–6855

[9] Bruggemann, M. and Neuberger, M.S. (1996) Strategies for expressing human antibody repertoires in transgenic mice. *Immunol. Today* 17, 391–397

[10] Green, L.L. *et al.* (1994) Antigen-specific human monoclonal antibodies from mice engineered with human Ig heavy and light chain YACs. *Nat. Genet.* 7, 13–21

[11] Lonberg, N. (2005) Human antibodies from transgenic animals. *Nat. Biotechnol.* 23, 1117–1125

[12] Lonberg, N. *et al.* (1994) Antigen-specific human antibodies from mice comprising four distinct genetic modifications. *Nature* 368, 856–859

[13] Dessain, S.K. *et al.* (2004) High efficiency creation of human monoclonal antibody-producing hybridomas. *J. Immunol. Methods* 291, 109–122

[14] Gustafsson, B. and Hinkula, J. (1994) Antibody production of a human EBV-transformed B cell line and its heterohybridoma and trioma cell line descendants in different culture systems. *Hum. Antibodies Hybridomas* 5, 98–104

[15] Li, J. *et al.* (2006) Human antibodies for immunotherapy development generated via a human B cell hybridoma technology. *Proc. Natl. Acad. Sci. U.S.A.* 103, 3557–3562

[16] Steinitz, M. *et al.* (1977) EB virus-induced B lymphocyte cell lines producing specific antibody. *Nature* 269, 420–422

[17] Winter, G. and Milstein, C. (1991) Man-made antibodies. *Nature* 349, 293–299

[18] Yu, X. *et al.* (2008) Neutralizing antibodies derived from the B cells of 1918 influenza pandemic survivors. *Nature* 455, 532–536

[19] Simmons, C.P. *et al.* (2007) Prophylactic and therapeutic efficacy of human monoclonal antibodies against H5N1 influenza. *PLoS Med.* 4, e178

[20] Traggiai, E. *et al.* (2004) An efficient method to make human monoclonal antibodies from memory B cells: potent neutralization of SARS coronavirus. *Nat. Med.* 10, 871–875

[21] Hoogenboom, H.R. (2005) Selecting and screening recombinant antibody libraries. *Nat. Biotechnol.* 23, 1105–1116

[22] Lowe, D. and Jermutus, L. (2004) Combinatorial protein biochemistry for therapeutics and proteomics. *Curr. Pharm. Biotechnol.* 5, 17–27

[23] Rajpal, A. *et al.* (2005) A general method for greatly improving the affinity of antibodies by using combinatorial libraries. *Proc. Natl. Acad. Sci. U.S.A.* 102, 8466–8471

[24] Steidl, S. *et al.* (2008) In vitro affinity maturation of human GM-CSF antibodies by targeted CDR-diversification. *Mol. Immunol.* 46, 135–144

[25] Babcook, J.S. *et al.* (1996) A novel strategy for generating monoclonal antibodies from single, isolated lymphocytes producing antibodies of defined specificities. *Proc. Natl. Acad. Sci. U.S.A.* 93, 7843–7848

[26] Jiang, X. *et al.* (2006) A novel strategy for generation of monoclonal antibodies from single B cells using rt-PCR technique and in vitro expression. *Biotechnol. Prog.* 22, 979–988

[27] Kantor, A.B. *et al.* (1995) Development of the antibody repertoire as revealed by single-cell PCR of FACS-sorted B-cell subsets. *Ann. N. Y. Acad. Sci.* 764, 224–227

[28] Lagerkvist, A.C. *et al.* (1995) Single, antigen-specific B cells used to generate Fab fragments using CD40-mediated amplification or direct PCR cloning. *Biotechniques* 18, 862–869

[29] Meijer, P.J. *et al.* (2006) Isolation of human antibody repertoires with preservation of the natural heavy and light chain pairing. *J. Mol. Biol.* 358, 764–772

[30] Smith, K. *et al.* (2009) Rapid generation of fully human monoclonal antibodies specific to a vaccinating antigen. *Nat. Protoc.* 4, 372–384

[31] Tiller, T. *et al.* (2009) Cloning and expression of murine Ig genes from single B cells. *J. Immunol. Methods* 350, 183–193

[32] Tiller, T. *et al.* (2008) Efficient generation of monoclonal antibodies from single human B cells by single cell RT-PCR and expression vector cloning. *J. Immunol. Methods* 329, 112–124

[33] Jin, A. *et al.* (2009) A rapid and efficient single-cell manipulation method for screening antigen-specific antibody-secreting cells from human peripheral blood. *Nat. Med.* 15, 1088–1092

[34] Love, J.C. *et al.* (2006) A microengraving method for rapid selection of single cells producing antigen-specific antibodies. *Nat. Biotechnol.* 24, 703–707

[35] Ogunniyi, A.O. *et al.* (2009) Screening individual hybridomas by microengraving to discover monoclonal antibodies. *Nat. Protoc.* 4, 767–782

[36] Tajiri, K. *et al.* (2007) Cell-microarray analysis of antigen-specific B-cells: single cell analysis of antigen receptor expression and specificity. *Cytometry A* 71, 961–967

[37] Tokimitsu, Y. *et al.* (2007) Single lymphocyte analysis with a microwell array chip. *Cytometry A* 71, 1003–1010

[38] Kuppers, R. *et al.* (1993) Tracing B cell development in human germinal centres by molecular analysis of single cells picked from histological sections. *EMBO J.* 12, 4955–4967

[39] Obiakor, H. *et al.* (2002) A comparison of hydraulic and laser capture microdissection methods for collection of single B cells, PCR, and sequencing of antibody VDJ. *Anal. Biochem.* 306, 55–62

[40] Wardemann, H. *et al.* (2003) Predominant autoantibody production by early human B cell precursors. *Science* 301, 1374–1377

[41] Battye, F.L. *et al.* (2000) Single cell sorting and cloning. *J. Immunol. Methods* 243, 25–32

[42] Herzenberg, L.A. *et al.* (2002) The history and future of the fluorescence activated cell sorter and flow cytometry: a view from Stanford. *Clin. Chem.* 48, 1819–1827

[43] Scheid, J.F. *et al.* (2009) A method for identification of HIV gp140 binding memory B cells in human blood. *J. Immunol. Methods* 343, 65–67

[44] Tickle, S. *et al.* (2009) High-throughput screening for high affinity antibodies. *J. Assoc. Lab. Automation* 14, 303–307

[45] Scheid, J.F. *et al.* (2009) Broad diversity of neutralizing antibodies isolated from memory B cells in HIV-infected individuals. *Nature* 458, 636–640

[46] Wrammert, J. *et al.* (2008) Rapid cloning of high-affinity human monoclonal antibodies against influenza virus. *Nature* 453, 667–671

[47] Kantor, A.B. *et al.* (1997) An unbiased analysis of V(H)-D-J(H) sequences from B-1a, B-1b, and conventional B cells. *J. Immunol.* 158, 1175–1186

[48] Kantor, A.B. *et al.* (1998) Construction of cDNA from single unstimulated mouse B lymphocytes: method and application to the study of expressed antibody repertoires in FACS-sorted murine B cell subsets. In *Handbook of Experimental Immunology* (Herzenberg, L.A., Blackwell, C., Herzenberg, L.A., eds), Blackwell pp. 13.1–13.6

[49] Coronella, J.A. *et al.* (2000) Amplification of IgG VH and VL (Fab) from single human plasma cells and B cells. *Nucleic Acids Res.* 28, E85

[50] Marks, J.D. *et al.* (1991) Oligonucleotide primers for polymerase chain reaction amplification of human immunoglobulin variable genes and design of family-specific oligonucleotide probes. *Eur. J. Immunol.* 21, 985–991

[51] Wang, X. and Stollar, B.D. (2000) Human immunoglobulin variable region gene analysis by single cell RT-PCR. *J. Immunol. Methods* 244, 217–225

[52] Liao, H.X. *et al.* (2009) High-throughput isolation of immunoglobulin genes from single human B cells and expression as monoclonal antibodies. *J. Virol. Methods* 158, 171–179

[53] Embleton, M.J. *et al.* (1992) In-cell PCR from mRNA: amplifying and linking the rearranged immunoglobulin heavy and light chain V-genes within single cells. *Nucleic Acids Res.* 20, 3831–3837

[54] Ozawa, T., Kishi, H. and Muraguchi, A. (2006) Amplification and analysis of cDNA generated from a single cell by 5′-RACE: application to isolation of antibody heavy and light chain variable gene sequences from single B cells. *Biotechniques* 40, 469–470 472, 474

[55] de Wildt, R.M. *et al.* (1999) Somatic insertions and deletions shape the human antibody repertoire. *J. Mol. Biol.* 294, 701–710

[56] Wilson, P.C. *et al.* (1998) Somatic hypermutation introduces insertions and deletions into immunoglobulin V genes. *J. Exp. Med.* 187, 59–70

[57] Brezinschek, H.P. *et al.* (1995) Analysis of the heavy chain repertoire of human peripheral B cells using single-cell polymerase chain reaction. *J. Immunol.* 155, 190–202

[58] Ehlich, A. and Kuppers, R. (1995) Analysis of immunoglobulin gene rearrangements in single B cells. *Curr. Opin. Immunol.* 7, 281–284

[59] Ghia, P. *et al.* (1996) Ordering of human bone marrow B lymphocyte precursors by single-cell polymerase chain reaction analyses of the rearrangement status of the immunoglobulin H and L chain gene loci. *J. Exp. Med.* 184, 2217–2229

[60] ten, B.E. *et al.* (1995) The status of Ig loci rearrangements in single cells from different stages of B cell development. *Int. Immunol.* 7, 1013–1019

[61] Seidl, K.J. *et al.* (1997) Frequent occurrence of identical heavy and light chain Ig rearrangements. *Int. Immunol.* 9, 689–702

[62] de Wildt, R.M. *et al.* (1999) Analysis of heavy and light chain pairings indicates that receptor editing shapes the human antibody repertoire. *J. Mol. Biol.* 285, 895–901

[63] Wardemann, H. and Nussenzweig, M.C. (2007) B-cell self-tolerance in humans. *Adv. Immunol.* 95, 83–110

[64] Isnardi, I. *et al.* (2008) IRAK-4- and MyD88-dependent pathways are essential for the removal of developing autoreactive B cells in humans. *Immunity* 29, 746–757

[65] Yurasov, S. *et al.* (2005) B-cell tolerance checkpoints in healthy humans and patients with systemic lupus erythematosus. *Ann. N. Y. Acad. Sci.* 1062, 165–174

[66] Yurasov, S. *et al.* (2006) Persistent expression of autoantibodies in SLE patients in remission. *J. Exp. Med.* 203, 2255–2261

[67] Samuels, J. *et al.* (2005) Impaired early B cell tolerance in patients with rheumatoid arthritis. *J. Exp. Med.* 201, 1659–1667

[68] Tsuiji, M. *et al.* (2006) A checkpoint for autoreactivity in human IgM+ memory B cell development. *J. Exp. Med.* 203, 393–400

[69] Koelsch, K. *et al.* (2007) Mature B cells class switched to IgD are autoreactive in healthy individuals. *J. Clin. Invest.* 117, 1558–1565

[70] Tiller, T. *et al.* (2007) Autoreactivity in human IgG+ memory B cells. *Immunity* 26, 205–213

[71] Manz, R.A. *et al.* (2005) Maintenance of serum antibody levels. *Annu. Rev. Immunol.* 23, 367–386

[72] Wu, X. *et al.* (2010) Rational design of envelope identifies broadly neutralizing human monoclonal antibodies to HIV-1. *Science*

[73] Poulsen, T.R. *et al.* (2007) Kinetic, affinity, and diversity limits of human polyclonal antibody responses against tetanus toxoid. *J. Immunol.* 179, 3841–3850

[74] Dohmen, S.E. *et al.* (2005) Production of recombinant Ig molecules from antigen-selected single B cells and restricted usage of Ig-gene segments by anti-D antibodies. *J. Immunol. Methods* 298, 9–20

[75] Tajiri, K. *et al.* (2010) Analysis of the epitope and neutralizing capacity of human monoclonal antibodies induced by hepatitis B vaccine. *Antiviral Res.*

[76] Mietzner, B. *et al.* (2008) Autoreactive IgG memory antibodies in patients with systemic lupus erythematosus arise from nonreactive and polyreactive precursors. *Proc. Natl. Acad. Sci. U.S.A.* 105, 9727–9732

[77] Casadevall, A. and Scharff, M.D. (1995) Return to the past: the case for antibody-based therapies in infectious diseases. *Clin. Infect. Dis.* 21, 150–161

[78] Newcombe, C. and Newcombe, A.R. (2007) Antibody production: polyclonal-derived biotherapeutics. *J. Chromatogr. B: Analyt. Technol. Biomed. Life Sci.* 848, 2–7