Int J Clin Exp Med 2016;9(10):18868-18882
www.ijcem.com /ISSN:1940-5901/IJCEM0032565

Review Article

Basic research and clinical application of immune repertoire sequencing

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Received May 21, 2016; Accepted August 11, 2016; Epub October 15, 2016; Published October 30, 2016

Abstract: The adaptive immune system uses several strategies to generate a repertoire of T- and B-cell antigen receptors (TCR/BCR) with sufficient diversity to recognize the wide array of potential pathogens. The Immunoglobulin and TCR repertoires are a mirror of the human immune system, which reflects processes caused by infections, cancer, autoimmunity and aging. The rapid advances in sequencing technologies have provided improvements in read length, throughput and cost. Nowadays, high-throughput sequencing (HTS) of lymphoid receptor genes is an emerging technology that could comprehensively assess the diversity of the immune system, and has been applied to vaccine development and efficacy assessment, biomarker discovery, minimal residual disease detection, autoimmune diseases, transplant rejection and tolerance. As biotechnology progresses, immune repertoire sequencing will applied more and more. The following review details some of the technologies and recent achievements in this field.

Keywords: T cell receptor, B cell receptor, Immune repertoire sequencing, clinical application

Introduction

The ability of the adaptive immune system to respond to any of the vast number of potential foreign antigens to which a person might be exposed relies on the highly polymorphic receptors expressed by B cells (immunoglobulins) and T cells (T-cell receptors [TCRs]). B-cell receptors (BCRs) are surface membrane immunoglobulins that can recognize antigens and have antigen-binding specificity. T lymphocytes are key mediators of adaptive immunity, which can recognize specifically antigen peptides presented by MHCs through TCRs on the cell surface. Each BCR comprises a pair of heavy and light chains. The heavy chain can be divided into a consistent region (C region), a variable region (V region), a transmembrane domain and a cytoplasmic domain, while the light chain only has a V region and C region. The V regions consist of a variable light (VL) domain and a variable heavy (VH) domain, which contain three complementary determining regions (CDR1, CDR2 and CDR3), respectively. Most TCRs comprise an α-chain and β-chain, while the rest consist of γ and δ-chains. Each chain can be divided into consistent region (C region), variable region (V region), transmembrane domain and cytoplasmic domain. The V region of the a and β-chains contains three hypervariable regions: CDR1, CDR2 and CDR3. The CDR3 loop is much more variable in length, sequence and structure [1], as it is generated by somatic recombination of variable (V), joining (J) and, in the case of the β-chain, also diversity (D) genes, termed V(D)J recombination. Random trimming and addition of non-template nucleotides at the junction sites (N-diversity mechanisms) greatly increases the diversity further [2, 3]. Theoretically, a repertoire of approximately 10^{18} different TCRs could be generated in humans. As CDR3 interacts most closely with the antigenic peptide, the diversity of CDR3 amino acid sequences provides a measure of T cell diversity in an antigen-selected T cell repertoire.

However, the immense theoretical combinatorial diversity represents a challenge to immu-

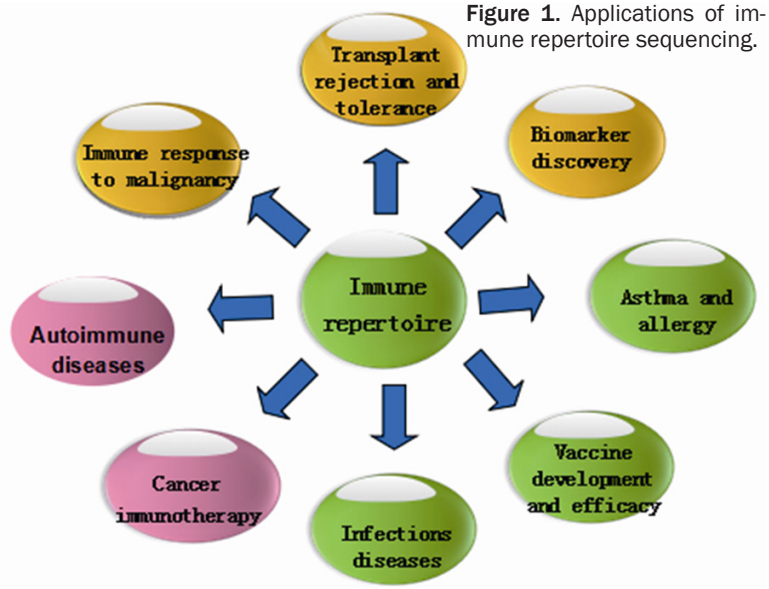

Figure 1. Applications of immune repertoire sequencing.

nology. Over the past two decades, several strategies have been developed to probe human TCR diversity. One strategy aimed to identify the presence of different TCR families using PCR or flow cytometry to determine the usage of different TCR variable (V) genes [4]. However, fluorescence-activated cell sorting (FACS) analysis is applicable to all the TCRAV and TCRBV segments, because specific monoclonal antibodies (mAbs) are not available for all TCRAVs and TCRBVs. Furthermore, FACS analysis requires a substantial number of cells to estimate the expression of TCRAV and TCRBV genes. Therefore, it is difficult to analyze a small number of T cells from inflammatory lesions. To overcome the disadvantage of FACS analysis, PCR based methods have been developed in many laboratories. However, different amplification efficiencies among individual primers and cross-reactivity between subfamilies have hampered the estimation of the precise frequency of individual TCRBV families [5]. A third strategy, called CDR3 size spectratyping, aims to determine the polyclonality of the repertoire using fluorescent primers to measure length variation of the CDR3 region within each TCR V family [6]. Spectratyping has been useful in documenting substantial [7]. However, spectratyping does not assess TCR CDR3 diversity at the sequence level, and cannot determine directly the number of distinct TCRs in a population of a and b T cells. Overall, as neither of these strategies can provide information about the relative frequency of each receptor in the population, they can only provide an estimate of repertoire complexity. Sanger sequencing can deliver single clone resolution. However, the enormous workload precludes detailed analysis by Sanger sequencing, which characterizes only highly dominant clones with biased frequency and leaves low-abundance clones aside. Nowadays, with the advent of next generation sequencing (NGS) technologies, it is possible to sequence millions of receptor clones representing the entire immune repertoire in a routine experiment. High-resolution is one of the main advantages of NGS and provides an opportunity to analyze clones with extremely low frequencies. A realistic analysis of the immune repertoire is crucial to understand the basic molecular mechanisms of adaptive immunity in health and disease, including the consequences of autoimmunity, infections, irradiation, immunosuppression and blood transplantation therapies, aging, and other conditions [8]. Nowadays, immune repertoire sequencing is applicable to, for example, vaccine development and efficacy assessment, biomarker discovery, minimal residual disease detection, autoimmune diseases, transplant rejection and tolerance (**Figure 1**). In the present article, we summarized the current knowledge in this field for a better understanding of the applied and basic research into immune repertoire sequencing.

Pathogenic mechanisms of complex diseases and the discovery of biomarkers

The TCR and BCR sequence repertoire of an individual represents a surrogate of their T and B-cell clonality status in health and disease, with the potential to provide new insights into the adaptive immune response, as well as to obtain a sufficient amount of sequences to provide biomarkers to assess disease risk, diagnosis or prognosis.

Infectious diseases

Cellular immunity is indispensable for combating numerous infections. The protective capaci-

ties of pathogen-specific T cells are defined not only by their phenotype and amount, but also by the TCR repertoire diversity allowing targeting of different pathogenic epitopes [9, 10]. Thus, in-depth clonotype analysis will contribute to a comprehensive understanding of infection pathogenesis and immunity. The first reports of TCR bias emerged in the early 1990s from studies of individuals exposed to Epstein-Barr virus (EBV) and influenza A virus [11, 12]. In 1991, Moss et al. [11] observed the first example of type IV TCR bias in humans. In this instance, HLA-A*0201-restricted CD8+ T-cell clones specific for the influenza virus matrix protein $(MP)_{58-66}$ peptide (GILGFVFTL[GIL]) were observed to encode highly homologous TRBV19/TRBJ2-7 TCRs. Since this initial study, many additional reports of TCR bias have surfaced in the setting of infectious diseases. A recent study demonstrated the presence of multiple biased TRBV families in recovered acute hepatitis B virus infection (AHI) subjects [13]. TRBV11, BV15 and BV20, especially from the CD8+ T-cell subset, might be relevant to the pathogenesis of subjects with AHI. In addition, the preferentially selected TRBV5.1 and BV20, with their relatively conserved CDR3 motifs, might be potential targets for personalized treatments of chronic HBV infection. In addition, Kim et al. [14] devised a deep sequencing-based approach to track the evolution of TCR repertoires after acute infection. They found that sustained TCR-pMHCII interactions are a key component of the memory T cell differentiation signal for CD4+ T cells.

Autoimmune diseases

It is widely believed that TCR bias is chiefly confined to foreign antigen-specific T-cell responses. However, it is often underappreciated that this phenomenon is also deeply entrenched in the cellular immune response to self. Many examples of TCR bias in autoimmunity have been published. Klarenbeek et al. [15] used an NGS protocol to profile quantitatively the T-cell repertoire in peripheral blood and multiple joints of patients with recent onset (early) or established rheumatoid arthritis (RA) to identify potential autoreactive clones. Their data showed clear oligoclonal expansions in the synovium, with great overlap between different joints. In contrast, the TCR repertoire in peripheral blood was polyclonal and there was hardly any overlap between the synovium and blood. This observation was in accordance with previous studies in type 1 diabetes mellitus (T1DM), which reported an extremely low frequency of pancreas-specific T cells in the blood (<0.001%) and enrichment for autoreactive T cells in the (T1DM) pancreas compared with the peripheral blood [16, 17]. These findings implied that analysis of clonality in blood samples might not be informative for the T-cell repertoire in the inflamed synovium. It is tempting to speculate that this retention is caused by the presence of specific (auto) antigens in the synovium. In addition, they addressed the question of whether the highly expanded clones (HECs) identified in the synovium showed similarities in their TCR sequences between different patients. They found that at the sequence and peptide level, there was no evidence of strong similarities of HECs between different patients, despite the presence of at least one DRB1 04-allele in at least nine of the 12 patients. This was confirmed in another study [18], which also reported that each patient had a unique repertoire, with little overlap among patients.

Tumour immunology

Recent work in tumour immunology has highlighted the need for quantitative, reproducible and simple measures of the host anti-tumor immune response, both to predict patient response to immunotherapy and to improve prognosis. Sherwood and co-workers demonstrated that ultra-deep TCR sequencing could determine the clonality of T cells directly in DNA extracted from tumor tissues [19]. This provided a powerful new tool to investigate the diversity and magnitude of T cell responses in tumors. However, a major barrier to the clinical assessment of the predictive power of these quantitative methods has been the suggestion that the intratumoral immune repertoire is inconsistent and so cannot be measured meaningfully with a single tissue sample. Subsequent to this, Gerlinger et al. [20] reported that the clonal composition of T cell populations could be heterogeneous across different regions of the same clear cell renal cell carcinoma (ccRCC), demonstrating intratumor heterogeneity of T cell immunity. Relevant to this investigation, Emerson et al. [21] used a multiplex PCR approach to amplify the TCRB CDR3 regions, followed by high-throughput sequencing (HTS)

to quantify the antigen receptor repertoire of multiple tumor punch biopsies from each ovarian carcinoma patient, as well as matched peripheral blood samples. They found that the T-cell response within ovarian carcinoma tumors was primarily homogeneous across the spatial extent of these large tumors, but peripheral blood samples showed TCRB repertoires that were quite distinct from the tumor tissue. In addition, type III and type IV TCR bias has been observed in tumor-associated antigens (TAA)-specific T cells from synovial sarcoma, melanoma, and prostate cancer patients [22]. These TRBV12-3/TRBJ2-1/TRAV17/TRAJ31-expressing T cells were found to be specific for the HLA-A*0201-restricted NY-ESO-1157-165 peptide. This TAA is of particular interest because it is expressed by numerous cancers, including the above malignancies, transitional cell carcinoma [23], lung and bladder cancer [24], and ovarian cancer [25]. Therefore, the HTS approach provides a useful tool to track T cell expansion kinetics, assessing immune competence and identifying antigen-specific T cell clones in patients with cancer. Understanding those is likely to aid the development of immunotherapy for the treatment of tumors.

Other immunity-related diseases

In addition, Wu et al. [26] reported that the TCR repertoire diversity in CD8+ Temra cells and CD45RO+ CD4+ cells of patients with Wiskott–Aldrich syndrome (WAS) was significantly skewed compared with that seen in age-matched control subjects. They used the Ds and H' diversity index to evaluate quantitatively the overall diversity of patients' TCR repertoires, and found that both the Ds and H' values of the patient group were lower than those of the control group in all T-cell subsets, which indicated that the TCR repertoires of the patients were less diverse than those of control subjects. These findings suggested that decreased and skewed TCR Vβ diversity may be involved in the pathogenesis of autoimmunity and immunodeficiency in patients with WAS. Moreover, to test the hypothesis that IgG4+ clones in idiopathic IgG4- related disease (IgG4-RD) are accompanied by a manifestation of IgG4- associated cholangitis (IAC), Maillette de Buy Wenniger et al. [27] used an NGS approach to screen the BCR repertoires. They observed highly abundant IgG4+ BCR clones in blood and tissue of patients with active IAC, which disappeared upon corticosteroid treatment, which suggested that specific B cell responses are pivotal to the pathogenesis of IAC. Relevant to this investigation, larger prospective cohorts are needed to verify the feasibility of using the dominant IgG4+ clones as a diagnostic marker for IgG4-RD. In addition, Rachael and co-workers reported recently that BCR sequencing in chronic lymphocytic leukemia (CLL) might provide an additional prognostic value for the disease [28].

Tracking and evaluating the disease treatment effect

High-throughput sequencing for minimal residual disease (MRD) quantification

T-lineage acute lymphoblastic leukemia/lymphoma (T-ALL) is an immature, aggressive, malignant T cell neoplasm that affects both pediatric and adult patients. Although there has been progress in treating T-ALL patients, with some achieving durable responses, a subset of these patients are over-treated because of an inability to sufficiently individualize clinical treatment. By contrast, others are inadequately treated and suffer disease relapse. Several studies have confirmed the importance of assessing MRD to predict clinical outcomes of patients [29, 30]. Current methodologies to monitor MRD in ALL include multiparametric flow cytometry (mpFC) and quantitative polymerase chain reaction (PCR)-based methods, which can detect recurrent and/or persistent disease with a sensitivity of 0.01% and 0.001%, respectively. However, these methods are operator and laboratory-dependent, and are difficult to implement in a uniform manner [31]. More importantly, the sensitivity of MRD monitoring methods needs to be improved. Recently, HTS of lymphoid receptor genes has been used to monitor lymphocyte diversity after adoptive immunotherapy using chimeric antigen receptor-modified T cells to treat chemotherapy-refractory chronic lymphocytic leukemia [32], as well as to monitor disease in B lymphoproliferative disorders [33]. In this regard, Wu and his colleagues compared HTS to mpFC for MRD assessment in patients with T-lineage ALL [34], and found that HTS of TCRB and TCRG identified MRD that was not detected by flow cytometry in a subset of cases (25 of 35 HTS compared with 13 of 35, respectively), which highlighted the potential of this technology to define

lower detection thresholds for MRD that could affect clinical treatment decisions. To further assess the suitability of HTS to monitor MRD in ALL, Faham et al. [35] compared its capacity to measure MRD to that of flow cytometry and allele-specific oligonucleotide (ASO)-PCR in follow-up samples from more than 100 patients with ALL. This study also demonstrated that HTS allows monitoring of the treatment response in ALL with great sensitivity and precision, and suggested that the sequencing assay sensitivity was limited only by the number of input cells and thus could detect residual disease at levels well below 1 in 1 million leukocytes (0.0001%). This was confirmed in another study, which also reported that HTS might facilitate routine MRD quantification in clinical trials. The authors applied the LymphoSIGHT method, an IGH-HTS MRD platform with a validated detection limit of 10^{-6} and a quantitative range above 10^{-5}, to predict relapse in 40 patients who underwent reduced-intensity allo-HCT for high-risk CLL. They found that disease-free survival was 86% in patients with MRD< 10^{-4} and 20% in those with MRD≥10^{-4} (P< 0.0001) with a median follow-up of 36 months [36]. Additionally, MRD was predictive of relapse at other time points, including 9, 18 and 24 months post-HCT. An MRD doubling time <12 months with a disease burden ≥10^{-5} was associated with relapse within 12 months of MRD assessment in 50% of patients, and within 24 months in 90% of patients. In summary, the universal applicability, sensitivity and capacity to capture clonal evolution of the HTS method described here, together with the results of comparisons with standard MRD assays in clinical samples, strongly support its potential as a next-generation MRD test for ALL.

Therapeutic efficacy assessment of infection diseases

The human immune system mounts continuous responses against viruses to prevent them from causing disease. Understanding this response might help to understand why the immune system does not clear these viruses and might help in preventive and therapeutic strategies. In this regard, chronic viral infections with extensive variation, such as human immunodeficiency virus (HIV) and hepatitis C, pose a great challenge for the cellular immune response [37, 38]. Numerous reports have documented changes in the TCR Vβ repertoire during HIV infection in relation to the effect of therapy and disease progression [39-41]. Successful antiretroviral therapy (ART) decreases viral loads and reduces the level of T cell activation. A recent study by Conrad et al. [42] determined the TCR repertoires of 14 HIV-specific CD8+ T cell responses from eight HIV-positive individuals before and after initiation of ART. They found decreased activation of CD4+ and CD8+ T cell populations and changes in memory subset distributions after initiation of ART. The TCR repertoire diversity decreased (P<0.0001), and this was accompanied by a reduction in the magnitude of HIV epitope-specific T cell responses (P=0.024) after ART. Despite this narrowing of the T cell response to HIV, the overall hierarchy of dominant T cell receptor clonotypes remained stable compared with that pre-ART, and these T cell populations retained a PD-1 high phenotype compared with subdominant clonotypes. These data suggested that the antigen burden might maintain TCR diversity and that dominant clonotypes are sensitive to antigens even after dramatic reductions after initiation of ART. Relevant to this investigation, many lines of evidence have suggested that HIV infection causes specific depletion of CD4-negative Vγ2Vδ2 T cells (a subset of γδ T cells) in patients with HIV disease [43]. Prolonged ART appears to reconstitute the Vγ2Vδ2 T cells [44]. Untreated HIV-positive patients with persistent low-level viremia had low levels of Vγ2Vδ2 T cells [45] and significant damage to their T-cell receptor (TCR) repertoire [43]. Chaudhry et al. [46] applied HTS for repertoire analysis to determine the mechanism for Vγ2Vδ2 T-cell reconstitution after long-term virus suppression. They found that prolonged HIV suppression with ART leads to reconstitution of the Vγ2Vδ2 T-cell subset deleted in HIV disease. Direct evidence for the repair of the T-cell receptor repertoire supports a view that treatment-associated immune reconstitution acts via new cell synthesis and not by expansion of residual cell populations. In addition, to track virus-specific CD4+ T cell repertoires after bacterial or acute viral infection, Kim and co-workers generated a single-chain TCR transgenic mouse that expressed the TCR α-chain cloned from the SMARTA TCR [47]. Subsequent to this, the authors employed a deep-sequencing-

based approach to track the evolution of TCR repertoires after acute infection [14]. They found that sustained TCR-pMHCII interactions were a key component of the memory T cell differentiation signal for CD4+ T cells.

Therapeutic efficacy assessment in autoimmune diseases

Patients with multiple sclerosis (MS) exhibit TCR repertoire alterations [48], with several expansions being found in the CSF or in MS brain lesions [49, 50]. The antibody natalizumab is an effective therapy for relapsing-remitting MS. However, natalizumab alters the cellular composition in the perivascular spaces and in the CSF, leading to an inhibitory effect on CNS immune surveillance [51]. This might increase the risk of progressive multifocal leukoencephalopathy (PML) caused by JC polyomavirus (JCV) [52, 53]. Relevant to this investigation, Warnke et al. [54] applied CDR3 spectratyping to assess the TCR repertoire in CSF and blood in 59 patients with relapsing-remitting MS treated with natalizumab for at least 18 months, five cases of natalizumab-associated PML, 17 age- and sex-matched patients with MS not treated with natalizumab, and 12 healthy controls. They found that patients with MS exhibited peripheral TCR repertoire expansions in their blood, which confirmed the results of several previous studies. However, it was worth noting that natalizumab therapy led to a reduction of Vβ elements with TCR repertoire expansions in peripheral venous blood T cells compared non-natalizumab-treated patients with MS. In addition, TCR repertoire restrictions observed in the CSF were most pronounced in patients with MS treated with natalizumab, which reflected an altered immune surveillance of the CNS and might contribute to an increased risk of developing PML. Based on these findings, natalizumab seems to induce a delayed and impaired peripheral expansion of antigen-specific T cells, whereas increased reconstitution of peripheral T-cell expansion following plasma exchange might trigger PML-immune reconstitution inflammatory syndrome (PML-IRIS). In conclusion, their data showed that treatment with natalizumab results in broader changes in the T-cell immune repertoire beyond lymphocyte migration.

Efficacy assessment of gene therapy

X-linked severe combined immunodeficiency (SCID-X1) is caused by mutations in the common cytokine receptor γ chain. Classically, these mutations lead to complete absence of functional T and natural killer cell lineages, as well as intrinsically compromised B cell function. Gene therapy offers a highly effective option for treatment of SCID-X1 when HLA-matched donors are unavailable, and provides long-term reconstitution of a polyclonal T cell repertoire that effectively restores clinical immunity. Gaspar et al. [55] applied immune repertoire technology to analyze the clinical outcome in ten children with molecularly defined SCID-X1 who underwent gene therapy at a median age of 10 months. They found that a functional polyclonal T cell repertoire was restored in all patients, and all the patients were alive after a median follow-up of 80 months (range, 54 to 107 months). Humoral immunity was only partially recovered, but was sufficient in some patients to allow for withdrawal of immunoglobulin replacement. There were no deaths, although one patient developed T-ALL as a result of vector-mediated insertional mutagenesis, and three patients developed antibiotic-responsive acute pulmonary infection after discontinuation of immunoglobulin replacement and/or antibiotic prophylaxis. In terms of immunological reconstitution, their findings were similar to those reported recently from another study. Hacein-Bey-Abina et al. [56] reported long-term follow-up (median, 108 months; range, 96 to 144 months) of nine patients with a similar pattern of T cell, B cell and NK cell recovery. Such data provide important independent evidence that gene therapy is clinically effective.

Applications of immune repertoire sequencing in post-transplant monitoring

Organ transplantation has become the preferred modality for the treatment of organ failure because of ongoing advances in transplant immunology and surgical techniques, and has led to improved survival, reduced healthcare costs, and significantly improvement the quality of life. However, viral infections are the major causes of morbidity and mortality in the post-operative period after solid organ transplantation [57]. T cells are crucial for control of viral infections. Therefore, qualitative and quantitative analyses of antiviral T cell immunity are beneficial for the successful management of transplant recipients, allowing personalized adjustment of immunosuppression. The main-

tenance and selection of CD8+ T-cell clones is pivotal in antiviral immune responses. Whether virus-specific CD8+ T-cell repertoires in response to herpes viruses in humans are indeed stable or do they show signs of flexibility over time? To investigate this, Klarenbeek et al. [58] combined tetramer sorting with quantitative NGS to study primary infections of human cytomegalovirus (hCMV) and Epstein Barr virus (EBV) in renal transplant recipients. For both viruses, they found that nearly all virus-specific CD8+ T-cell clones that appeared during the early phase of infection were maintained at high frequencies during the 5-year follow-up and hardly any new anti-viral clones appeared. In the viral latency phase, EBV and hCMV-specific clones remained amongst the most abundant clones in the CD8+ T-cell population, illustrating the relentless and stable continuation of the responses to these herpes viruses by the immune system. In addition, Dziubianau and his colleagues performed TCR repertoire analysis of various specimens in clinical settings, including polyomavirus BK (BKV) reactivation, CMV and acute cellular allograft rejection, and demonstrated a critical contribution of NGS-based clonotype analysis for differential diagnosis and further therapeutic decisions in clinical cases of post-transplant allograft impairment [57].

In addition, immune repertoire sequencing technology also has a great ability to assess immune reconstitution after allogeneic hematopoietic cell transplantation. Hematopoietic stem cell transplantation (HSCT) is an effective therapy for a wide range of hematological and non-hematological malignancies [59]. The benefits of HSCT are in a large part related to the development of effective immune responses against the underlying malignancy. In clinical trials, HSCT has been evaluated for severe autoimmunity as a method to “reset” the immune system and produce a new, non-autoimmune repertoire. However, before transplantation, patients undergo conditioning with chemotherapy, with or without irradiation, which results in severe immunodeficiency that can take months or years to restore, particularly in the T cell compartment [60, 61]. Restricted TCR diversity and delayed T cell recovery after HSCT predispose patients to infection and cancer relapse [62, 63]. Accurate and quantitative determination of the relationship of regenerated T cells to the baseline repertoire has been difficult to assess. In this context, Muraro et al. [64] used high-throughput deep TCRβ chain sequencing to assess millions of sorted CD8+ and CD4+ T cells from each multiple sclerosis patient before and after HSCT. They found that HSCT has distinct effects on CD8+ and CD4+ T cell repertoires. In the case of CD8+ T cells, dominant CD8+ clones were not effectively removed, and the reconstituted CD8+ repertoire was created by clonal expansion of cells that were present before treatment. By contrast, dominant CD4+ TCR clones present before treatment were undetectable following reconstitution, and patients largely developed a new repertoire. Moreover, it was noted that patients who failed to respond to treatment had a less diverse T cell repertoire early during the reconstitution process, underpinning the notion that repertoire complexity is critical for the reestablishment of immune tolerance. A similar study by van Heijst et al., [65] combined 5’ rapid amplification of complementary DNA ends PCR with deep sequencing to quantify TCR diversity in 28 recipients of allo-HSCT using a single oligonucleotide pair. They found that after 6 months, cord blood-graft recipients approximated the TCR diversity of healthy individuals, whereas recipients of T cell-depleted peripheral-blood stem cell grafts had 14-fold and 28-fold lower CD8+ and CD4+ T cell diversities, respectively. After 12 months, these deficiencies had improved for the CD4+, but not for the CD8+ T cell compartment. These findings indicated that there was substantial variability in the rate of recovery between different stem cell sources. In addition, the kinetics of B and T cell immune recovery are also affected by many pre and post-transplant factors. Considering this, Atar Lev and co-workers enrolled a unique group of patients (recombination activating gene 2 (RAG-2)-deficient severe combined immunodeficiency (SCID) patients) with profoundly depleted baseline B and T cell immunity to track the kinetics of B and T cell immune recovery after BMT [66]. They showed that the early peripheral presence of newly produced B and T lymphocytes from their production and maturation sites after BMT suggested donor stem cell origin rather than peripheral expansion. Although we could not explain this discrepancy, it might depend on the stage of cell differentiation, the cell type and the experimental methods; these observations need fur-

ther investigation. In HSCT, it is also worth noting that immune reactivity against the recipient in the form of acute graft-versus-host disease (aGVHD) is a leading cause of morbidity and mortality. For the 30% to 45% of patients that develop aGVHD, more than half develop gastrointestinal (GI) tract manifestations. Symptoms of GI aGVHD include anorexia, nausea, abdominal pain, diarrhea and hemorrhage [67]. The severity of these symptoms is a predictor of poor survival. Meyer et al. [18] analyzed the clonal TCRβ repertoire in patients with GI GVHD and found that the T-cell repertoire was more similar at different sites in the GI tracts of patients with severe treatment refractory GI GVHD compared with patients with mild, treatment-responsive aGVHD or with aGVHD-like symptoms without an identified cause. In addition, no clones were shared between all patients with aGVHD. One interpretation of this finding may be that there exists no universal aGVHD allogeneic antigen; however, it is also possible that multiple TCRβ CDR3 sequences may recognize the same antigen. Their study was on a small group, and we think that evaluating more patients who share HLA alleles could yield dominant shared clonotypes and other reproducible patterns. Based on these findings, it is likely that HTS technology would provide unprecedented views of the T cell repertoire recovery after HSCT and may identify patients at high risk of infection, GVHD or relapse.

Immune repertoire sequencing in vaccine design and antibody screening

Vaccine design

Large-scale repertoire analysis of immune receptors can provide powerful results. This technology may aid the development and discovery of vaccines and antibody therapeutics, and help us to gain a deeper understanding of the humoral response. A major challenge for vaccine development in the 21st century has been to identify immunogens that can elicit antibodies (Abs) capable of neutralizing diverse strains of HIV [68, 69]. Current vaccines that protect against a variety of pathogens most often use identical immunogens for priming and boosting, demonstrating that the elicited high-affinity, somatically mutated protective Abs and their non-mutated germline precursors can be engaged by the same structures. However, the predicted non-mutated germline ancestors of extensively mutated HIV-neutralizing Abs recognize HIV only weakly or not at all [70, 71], and this may in part explain why HIV envelope glycoprotein-based vaccines fail to induce broadly protective responses. Deep sequencing technology has the potential to aid these efforts by identifying efficiently bona fide human germline Igs as candidate precursors to any Ab of interest within multiple human repertoires. To demonstrate the potential utility of this new technology to informed, rational vaccine design, Larimore et al. [72] searched their germline IgH data for putative precursors of the membrane-proximal external region (MPER)-specific HIV-neutralizing Ab 4E10. They found 292 candidate precursors across four individuals from among 343,225 unique IgH rearrangements. These putative 4E10 IgH precursors represented 0.02~0.12% of unique IgH rearrangements from each individual. Moreover, their results indicated that although the closest germline 4E10H precursors were not shared between individuals, more distantly related candidate germline 4E10 precursors could be identified in multiple human germline Ig repertoires, suggesting the possibility that a priming immunogen could be developed to engage these precursors reproducibly in most individuals. Thus, their data demonstrated the potential utility of HST technology for vaccine development. Similarly, a recent study illustrated the usefulness and application of this methodology in the analysis of the human TR repertoire response towards a model immune challenge, the H1N1 vaccine [73]. In addition, Thomas et al. [74] used HTS to assess comprehensively how the naïve epitope-specific CD8+ cytotoxic T lymphocyte (CTL) repertoire translates to that found following an influenza-virus-specific immune response. They found that epitope-specific TCR use in an antiviral immune response was the consequence of a complex interplay between the naïve cytotoxic T lymphocyte precursor pool and extrinsic (likely antigen driven) influences, the contribution of which varied in an epitope-specific fashion. Moreover, work carried out by Zhu et al. reported that dermal-epidermal junction (DEJ) CD8aa+ T cells were tissue-resident cells that seemed to have a fundamental role in immune surveillance and in initial containment of HSV-2 reactivation in human peripheral tissue, and provided evidence that DEJ CD8aa+ T cells might mediate a

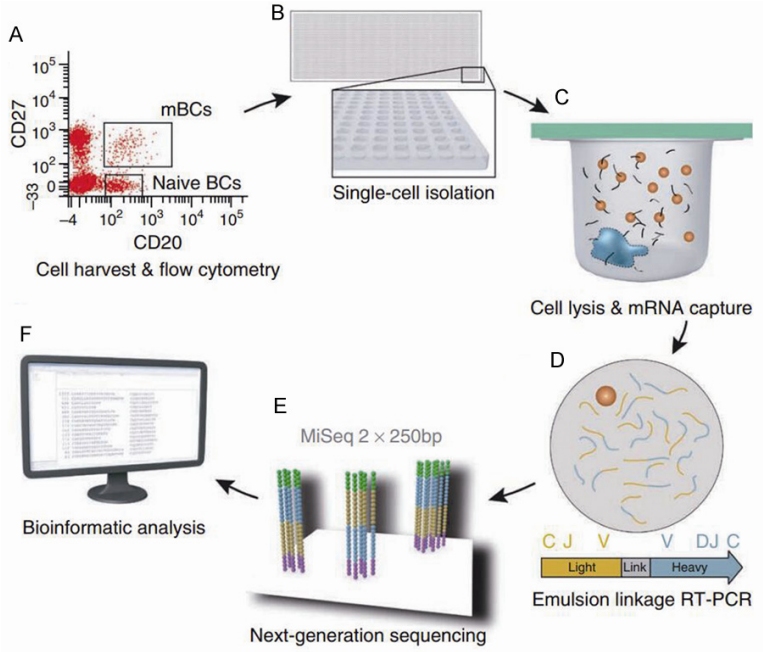

Figure 2. Overview of the high-throughput methodology for paired VH:VL antibody repertoire analysis. A. B-cell populations are sorted for desired phenotype (e.g., memory B cells, naive BCs, naive B cells, mBCs). B. Single cells are isolated by random settling into 125-pl wells (56-μm diameter) printed in polydimethylsiloxane (PDMS) slides the size of a standard microscope slide (1.7×10^5 wells/slide); 2.8-μm poly (dT) microbeads are also added to the wells (average 55 beads/well). C. Wells are sealed with a dialysis membrane and equilibrated with lysis buffer to lyse cells and anneal VH and VL mRNAs to poly (dT) beads (blue figure represents a lysed cell, orange circles depict magnetic beads, black lines depict mRNA strands; see Supplementary Video 1). D. Beads are recovered and emulsified for cDNA synthesis and linkage PCR to generate an ~850-base pair VH:VL cDNA product. E. Next-generation sequencing is performed to sequence the linked strands. F. Bioinformatic processing is used to analyze the paired VH:VL repertoire [88].

effective and rapid peripheral immunity to control frequent HSV antigen exposure in the human genital tract and skin [75]. Therefore, eliciting CD8aa+ T cells might be a critical component to develop effective vaccines against skin and mucosal infections.

Antibody production platform

After exposure to a foreign antigen, the mammalian humoral immune response generates a diverse repertoire of antibodies through changes in the genome of B cells by V(D)J gene recombination, gene conversion (in chicken and rabbit) and somatic hypermutation [76]. Each B-cell clone that undergoes this process contributes a specific monoclonal antibody to the diverse polyclonal response that is critical to fending off the infection. Antibodies are reagents valued for their ability to bind molecular surfaces with specificity and high-affinity. Antibodies and related products represent the fastest growing class of therapeutic agents [77, 78]. A variety of in vitro approaches have been developed to generate libraries to identify antibodies against numerous targets [79]. Some of these alternative strategies-involving single-cell sorting and molecular cloning, B-cell immortalization or phage display-have become increasingly effective; however, the antibodies they generate do not necessarily represent the actual antibody repertoire found in circulation and their production is often time consuming and labor intensive [80, 81]. In this regard, DNA sequencing has played a vital role in our understanding of antibody structure, function and diversification by the immune system [82]. It was instrumental in the identification of antibody variable and hypervariable regions, and increased our understanding of the manner in which the Ig repertoire is generated [83]. Initially, Sanger DNA sequencing was used to identify sequences of interest. This posed major limitations because only a minute fraction of the library was actually sampled; therefore, its content received only a superficial evaluation. Remarkably, HTS approaches are much better suited to library evaluation. Although massively parallel sequencing instrumentation was not even commercially available until a few years ago, we have seen a rapid expansion of HTS, reflecting a dynamic technology transition in this field [84]. Larman et al. [85] have developed an antibody production platform that incorporates seamless integration with short-read DNA sequencing technology. In addition, Weinstein and co-workers used the 454 GS FLX high-throughput pyrosequencing technology to characterize the antibody repertoire of zebrafish by analyzing CDR3 sequences of the heavy chain [86]. In their

study, HTS technology provided insight into the breadth of the expressed antibody repertoire and the immunological diversity at the level of an individual organism. In addition, a recent study by Cheung et al. [87] demonstrated a proteomics approach that leverages the strengths of two technologies, liquid chromatography-tandem mass spectrometry (LC-MS/MS) and NGS, for antibody discovery. Using this technology, they isolated monoclonal antibodies for five antigens from the sera of immunized mice and rabbits. This approach avoids the practical and technical challenges of existing methods, such as attrition during in vitro culturing of B cells, the need to construct and pan phage libraries or to extensively screen thousands of clones. However, this approach disrupts the cognate pairing of heavy and light chains, and thus cannot provide information about the identity of immune receptor pairs encoded by individual T or B-lymphocytes. DeKosky et al. [88] described a way to retain this pairing information. The experimental procedure is shown in **Figure 2**. In their approach, single B cells (>5 × 10^4 capacity per experiment) are deposited in a high-density microwell plate (125 pl/well) and lysed in situ. mRNA is then captured on magnetic beads, reverse transcribed and amplified by emulsion VH:VL linkage PCR. The linked transcripts are analyzed by Illumina HTS. They applied this technology to sequence the repertoire of three human cell subsets: peripheral blood IgG+ B cells, peripheral plasmablasts isolated after tetanus toxoid immunization and memory B cells isolated after seasonal influenza vaccination, to discover antigen-specific, high-affinity human antibodies. Overall, as the HTS platforms mature and evolve further, this approach will undoubtedly become widely integrated into synthetic antibody research and discovery programs. This will enable low-cost production of high quality synthetic antibodies.

Conclusions

In summary, HTS of immunoglobulin and TCR sequences offers a number of opportunities to expand our knowledge of human biology and medicine. Realistic analysis of the TCR CDR3 diversity is crucial to understand the basic molecular mechanisms of adaptive immunity in health and disease, including the consequences of autoimmunity, infections, irradiation, immunosuppression and blood transplantation therapies, aging and other conditions. Furthermore, this knowledge could be used in biomarker discovery, efficacy assessment and vaccine development. In this regard, a larger-scale and longer-term study is required to confirm the efficacy and safety of vaccines, and the sensitivity and specificity of biomarkers. In addition, as much as this technology presents an opportunity, it brings with it major challenges in data storage and data analysis. We need to consider the human ability to store, view and produce meaning from these data. Therefore, new databases, computational algorithms and software for the analysis of whole repertoires are required urgently. More importantly, potential errors during the sequencing process may skew the interpretation [89]. The enormous quantity of reads generated by NGS technologies necessitates cautious interpretation. Overall, we believe that with ongoing development of immune repertoire sequencing, scientific discoveries in TCR/BCR repertoire studies will form the basis for new clinical implications in personalized medicine and will provide a deeper understanding of immune behavior and the immune response.

Acknowledgements

This work was supported by funds received from the National Natural Science Foundation of China (No. 81271810, 81571953), 12-5 state S&T Projects for infectious diseases (2012ZX10002-007), Doctoral Fund of Ministry of Education of China (20120101110009), and Zhejiang medical science and technology project (2015118507).

Disclosure of conflict of interest

None.

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References

[1] Morea V, Tramontano A, Rustici M, Morea V, Tramontano A, Rustici M, Chothia C, Lesk AM. Conformations of the third hypervariable region in the VH domain of immunoglobulins. J Mol Biol 1998; 275: 269-294.

[2] Pannetier C, Cochet M, Darche S, Casrouge A, Zöller M, Kourilsky P. The sizes of the CDR3 hypervariable regions of the murine T-cell receptor beta chains vary as a function of the recombined germ-line segments. Proc Natl Acad Sci U S A 1993; 90: 4319-4323.

[3] Cabaniols JP, Fazilleau N, Casrouge A, Kourilsky P, Kanellopoulos JM. Most alpha/beta T cell receptor diversity is due to terminal deoxynucleotidyl transferase. J Exp Med 2001; 194: 1385-1390.

[4] Langerak AW, van Den Beemd R, Wolvers-Tettero IL, Boor PP, van Lochem EG, Hooijkaas H, van Dongen JJ. Molecular and flow cytometric analysis of the Vβ repertoire for clonality assessment in mature TCR αβ T-cell proliferations. Blood 2011; 98: 165-173.

[5] Diu A, Romagne F, Genevee C, Rocher C, Bruneau JM, David A, Praz F, Hercend T. Fine specificity of monoclonal antibodies directed at human T cell receptor variable regions: comparison with oligonucleotide-deriven amplification for evaluation of Vβ expression. Eur J Immunol 1993; 23: 1422-1429.

[6] Gorski J, Yassai M, Zhu X, Kissela B, Keever C, Flomenberg N. Circulating T cell repertoire complexity in normal individuals and bone marrow recipients analyzed by CDR3 size spectratyping. Correlation with immune status. J Immunol 1994; 152: 5109-5119.

[7] Verfuerth S, Peggs K, Vyas P, Barnett L, O'Reilly RJ, Mackinnon S. Longitudinal monitoring of immune reconstitution by CDR3 size spectratyping after T-cell-depleted allogeneic bone marrow transplant and the effect of donor lymphocyte infusions on T-cell repertoire. Blood 2000; 95: 3990-3995.

[8] Kedzierska K, La Gruta NL, Stambas J, Turner SJ, Doherty PC. Tracking phenotypically and functionally distinct T-cell subsets via T-cell repertoire diversity. Mol Immunol 2008; 45: 607-618.

[9] Messaoudi I, Guevara Patino JA, Dyall R, LeMaoult J, Nikolich-Zugich J. Direct link between mhc polymorphism, T cell avidity, and diversity in immune defense. Science 2002; 298: 1797-1800.

[10] Wang GC, Dash P, McCullers JA, Doherty PC, Thomas PG. T cell receptor alphabeta diversity inversely correlates with pathogen-specific antibody levels in human cytomegalovirus infection. Sci Transl Med 2012; 4: 128-142.

[11] Moss PA, Moots RJ, Rosenberg WM, Rowland-Jones SJ, Bodmer HC, McMichael AJ, Bell JI. Extensive conservation of alpha and beta chains of the human T-cell antigen receptor recognizing HLA-A2 and influenza A matrix peptide. Proc Natl Acad Sci U S A 1991; 88: 8987-8990.

[12] Argaet VP, Schmidt CW, Burrows SR, Silins SL, Kurilla MG, Doolan DL, Suhrbier A, Moss DJ, Kieff E, Sculley TB, Misko IS. Dominant selection of an invariant T cell antigen receptor in response to persistent infection by Epstein-Barr virus. J Exp Med 1994; 180: 2335-2340.

[13] Yang J, Chen J, He J, Xie Y, Zhu Y, Cao H, Li L. Profiling the repertoire of T-cell receptor betα-chain variable genes in peripheral blood lymphocytes from subjects who have recovered from acute hepatitis B virus infection. Cell Mol Immunol 2014; 11: 332-342.

[14] Kim C, Wilson T, Fischer KF, Williams MA. Sustained interactions between T cell receptors and antigens promote the differentiation of CD4+ memory T cells. Immunity 2013; 39: 508-520.

[15] Klarenbeek PL, de Hair MJ, Doorenspleet ME, van Schaik BD, Esveldt RE, van de Sande MG, Cantaert T, Gerlag DM, Baeten D, van Kampen AH, Baas F, Tak PP, de Vries N. Inflamed target tissue provides a specific niche for highly expanded T-cell clones in early human autoimmune disease. Ann Rheum Dis 2012; 71: 1088-1093.

[16] Roep BO. The role of T-cells in the pathogenesis of type 1 diabetes: from cause to cure. Diabetologia 2003; 46: 305-321.

[17] Velthuis JH, Unger WW, van der Slik AR, Duinkerken G, Engelse M, Schaapherder AF, Ringers J, van Kooten C, de Koning EJ, Roep BO. Accumulation of autoreactive effector T cells and allo-specific regulatory T cells in the pancreas allograft of a type 1 diabetic recipient. Diabetologia 2009; 52: 494-503.

[18] Meyer EH, Hsu AR, Liliental J, Löhr A, Florek M, Zehnder JL, Strober S, Lavori P, Miklos DB, Johnson DS, Negrin RS. A distinct evolution of the T-cell repertoire categorizes treatment refractory gastrointestinal acute graft-versus-host disease. Blood 2013; 121: 4955-4962.

[19] Sherwood AM, Emerson RO, Scherer D, Habermann N, Buck K, Staffa J, Desmarais C, Halama N, Jaeger D, Schirmacher P, Herpel E, Kloor M, Ulrich A, Schneider M, Ulrich CM, Robins H. Tumor-infiltrating lymphocytes in colorectal tumors display a diversity of T cell receptor sequences that differ from the T cells in adjacent mucosal tissue. Cancer Immunol Immunother 2013; 62: 1453-1461.

[20] Gerlinger M, Quezada SA, Peggs KS, Furness AJ, Fisher R, Marafioti T, Shende VH, McGranahan N, Rowan AJ, Hazell S, Hamm D, Robins HS, Pickering L, Gore M, Nicol DL, Larkin J, Swanton C. Ultra-deep T cell receptor sequencing reveals the complexity and intratumour heterogeneity of T cell clones in renal cell carcinomas. J Pathol 2013; 231: 424-432.

[21] Emerson RO, Sherwood AM, Rieder MJ, Guenthoer J, Williamson DW, Carlson CS, Drescher CW, Tewari M, Bielas JH, Robins HS. High-throughput sequencing of T-cell receptors reveals a homogeneous repertoire of tumour-infiltrating lymphocytes in ovarian cancer. J Pathol 2013; 231: 433-440.

[22] Le Gal FA, Ayyoub M, Dutoit V, Widmer V, Jäger E, Cerottini JC, Dietrich PY, Valmori D. Distinct structural TCR repertoires in naturally occurring versus vaccine-induced CD8+ T-cell responses to the tumor-specific antigen NY-ESO-1. J Immunother 2005; 28: 252-257.

[23] Kurashige T, Noguchi Y, Saika T, Ono T, Nagata Y, Jungbluth A, Ritter G, Chen YT, Stockert E, Tsushima T, Kumon H, Old LJ, Nakayama E. Ny-ESO-1expression and immunogenicity associated with transitional cell carcinoma: correlation with tumor grade. Cancer Res 2001; 61: 4671-4674.

[24] Jungbluth AA, Chen YT, Stockert E, Busam KJ, Kolb D, Iversen K, Coplan K, Williamson B, Altorki N, Old LJ. Immunohis-tochemical analysis of NY-ESO-1 antigen expression in normal and malignant human tissues. Int J Cancer 2001; 92: 856-860.

[25] Odunsi K, Jungbluth AA, Stockert E, Qian F, Gnjatic S, Tammela J, Intengan M, Beck A, Keitz B, Santiago D, Williamson B, Scanlan MJ, Ritter G, Chen YT, Driscoll D, Sood A, Lele S, Old LJ. NY-ESO-1 and LAGE-1 cancer-testis antigens are potential targets for immunotherapy in epithelial ovarian cancer. Cancer Res 2003; 63: 6076-6083.

[26] Wu J, Liu D, Tu W, Song W, Zhao X. T-cell receptor diversity is selectively skewed in T-cell populations of patients with Wiskott-Aldrich syndrome. J Allergy Clin Immunol 2015; 135: 209-216.

[27] Maillette de Buy Wenniger LJ, Doorenspleet ME, Klarenbeek PL, Verheij J, Baas F, Elferink RP, Tak PP, de Vries N, Beuers U. IgG4+ Clones Identified by Next-Generation Sequencing Dominate the B-Cell Receptor Repertoire in IgG4-Associated Cholangitis. Hepatology 2013; 57: 2390-2398.

[28] Bashford-Rogers RJ, Palser AL, Huntly BJ, Rance R, Vassiliou GS, Follows GA, Kellam P. Network properties derived from deep sequencing of human B-cell receptor repertoires delineate B-cell populations. Genome Res 2013; 23: 1874-84.

[29] Flohr T, Schrauder A, Cazzaniga G, Panzer-Grümayer R, van der Velden V, Fischer S, Stanulla M, Basso G, Niggli FK, Schäfer BW, Sutton R, Koehler R, Zimmermann M, Valsecchi MG, Gadner H, Masera G, Schrappe M, van Dongen JJ, Biondi A, Bartram CR; International BFM Study Group (I-BFM-SG). Minimal residual disease-directed risk stratification using real-time quantitative PCR analysis of immunoglobulin and T-cell receptor gene re-arrangements in the international multicenter trial AIEOP-BFM ALL 2000 for childhood acute lymphoblastic leukemia. Leukemia 2008; 22: 771-782.

[30] Schrappe M, Valsecchi MG, Bartram CR, Schrauder A, Panzer-Grümayer R, Möricke A, Parasole R, Zimmermann M, Dworzak M, Buldini B, Reiter A, Basso G, Klingebiel T, Messina C, Ratei R, Cazzaniga G, Koehler R, Locatelli F, Schäfer BW, Aricò M, Welte K, van Dongen JJ, Gadner H, Biondi A, Conter V. Late MRD response determines relapse risk overall and in subsets of childhood T-cell ALL: Results of the AIEOP-BFM-ALL 2000 study. Blood 2011; 118: 2077-2084.

[31] van der Velden VH, Panzer-Grümayer ER, Cazzaniga G, Flohr T, Sutton R, Schrauder A, Basso G, Schrappe M, Wijkhuijs JM, Konrad M, Bartram CR, Masera G, Biondi A, van Dongen JJ. Optimization of PCR-based minimal residual disease diagnostics for childhood acute lymphoblastic leukemia in a multi-center setting. Leukemia 2007; 21: 706-713.

[32] Kalos M, Levine BL, Porter DL, Katz S, Grupp SA, Bagg A, June CH. T cells with chimeric antigen receptors have potent antitumor effects and can establish memory in patients with advanced leukemia. Sci Transl Med 2011; 3: 95ra73.

[33] Boyd SD, Marshall EL, Merker JD, Maniar JM, Zhang LN, Sahaf B, Jones CD, Simen BB, Hanczaruk B, Nguyen KD, Nadeau KC, Egholm M, Miklos DB, Zehnder JL, Fire AZ. Measurement and clinical monitoring of human lymphocyte clonality by massively parallel VDJ pyrosequencing. Sci Transl Med 2005; 1: 12-23.

[34] Wu D, Sherwood A, Fromm JR, Winter SS, Dunsmore KP, Loh ML, Greisman HA, Sabath DE, Wood BL, Robins H. High-Throughput Sequencing Detects Minimal Residual Disease in Acute T Lymphoblastic Leukemia. Sci Transl Med 2012; 4: 134-163.

[35] Faham M, Zheng J, Moorhead M, Carlton VE, Stow P, Coustan-Smith E, Pui CH, Campana D. Deep sequencing approach for minimal residual disease detection in acute lymphoblastic leukemia. Blood 2012; 120: 5173-5180.

[36] Logan AC, Zhang B, Narasimhan B, Carlton V, Zheng J, Moorhead M, Krampf MR, Jones CD, Waqar AN, Faham M, Zehnder JL, Miklos DB. Minimal residual disease quantification using consensus primers and high-throughput IGH sequencing predicts post-transplant relapse in chronic lymphocytic leukemia. Leukemia 2013; 27: 1659-1665.

[37] Walker BD, Goulder PJ. AIDS: escape from the immune system. Nature 2000; 407: 313-314.
[38] Bowen DG, Walker CM. Adaptive immune responses in acute and chronic hepatitis C virus infection. Nature 2005; 436: 946-952.
[39] Douek DC, Brenchley JM, Betts MR, Ambrozak DR, Hill BJ, Okamoto Y, Casazza JP, Kuruppu J, Kunstman K, Wolinsky S, Grossman Z, Dybul M, Oxenius A, Price DA, Connors M, Koup RA. HIV preferentially infects HIV-specific CD4+ T cells. Nature 2002; 417: 95-98.
[40] Gorochov G, Neumann AU, Kereveur A, Parizot C, Li T, Katlama C, Karmochkine M, Raguin G, Autran B, Debré P. Perturbations of CD4+ and CD8+ T-cell repertoires during progression to AIDS and regulation of the CD4+ repertoire during antiviral therapy. Nat Med 1998; 4: 215-221.
[41] Halapi E, Jeddi-Tehrani M, Blücher A, Andersson R, Rossi P, Wigzell H, Grunewald J. Diverse T-cell receptor CDR3 length patterns in human CD4 and CD8 T lymphocytes from newborns and adults. Scand J Immunol 1999; 49: 149-154.
[42] Conrad JA, Ramalingam RK, Duncan CB, Smith RM, Wei J, Barnett L, Simons BC, Lorey SL, Kalams SA. Antiretroviral Therapy Reduces the Magnitude and T Cell Receptor Repertoire Diversity of HIV-Specific T Cell Responses without Changing T Cell Clonotype Dominance. J Virol 2012; 86: 4213-4221.
[43] Hebbeler AM, Propp N, Cairo C, Li H, Cummings JS, Jacobson LP, Margolick JB, Pauza CD. Failure to restore the Vgamma2-Jgamma1.2 repertoire in HIV-infected men receiving highly active antiretroviral therapy (HAART). Clin Immunol 2008; 128: 349-357.
[44] Bordon J, Evans PS, Propp N, Davis CE Jr, Redfield RR, Pauza CD. Association between longer duration of HIV-suppressive therapy and partial recovery of the V gamma 2 cell receptor repertoire. J Infect Dis 2004; 189: 1482-1486.
[45] Boudova S, Li H, Sajadi MM, Redfield RR, Pauza CD. Impact of persistent HIV replication on CD4 negative Vgamma2 Vdelta2 T cells. J Infect Dis 2012; 205: 1448-1455.
[46] Chaudhry S, Cairo C, Venturi V, Pauza CD. The γδ T-cell receptor repertoire is reconstituted in HIV patients after prolonged antiretroviral therapy. AIDS 2013; 27: 1557-1562.
[47] Oxenius A, Bachmann MF, Zinkernagel RM, Hengartner H. Virus-specific MHC-class II-restricted TCR-transgenic mice: effects on humoral and cellular immune responses after viral infection. Eur J Immunol 1998; 28: 390-400.
[48] Laplaud DA, Ruiz C, Wiertlewski S, Brouard S, Berthelot L, Guillet M, Melchior B, Degauque N, Edan G, Brachet P, Damier P, Soulillou JP. Blood T-cell receptor beta chain transcriptome in multiple sclerosis: characterization of the T cells with altered CDR3 length distribution. Brain 2004; 127: 981-995.
[49] Skulina C, Schmidt S, Dornmair K, Babbe H, Roers A, Rajewsky K, Wekerle H, Hohlfeld R, Goebels N. Multiple sclerosis: brain-infiltrating CD8+ T cells persist as clonal expansions in the cerebrospinal fluid and blood. Proc Natl Acad Sci U S A 2004; 101: 2428-2433.
[50] Junker A, Ivanidze J, Malotka J, Eiglmeier I, Lassmann H, Wekerle H, Meinl E, Hohlfeld R, Dornmair K. Multiple sclerosis: T-cell receptor expression in distinct brain regions. Brain 2007; 130: 2789-2799.
[51] del Pilar Martin M, Cravens PD, Winger R, Frohman EM, Racke MK, Eagar TN, Zamvil SS, Weber MS, Hemmer B, Karandikar NJ, Kleinschmidt-DeMasters BK, Stüve O. Decrease in the numbers of dendritic cells and CD41 T cells in cerebral perivascular spaces due to natalizumab. Arch Neurol 2008; 65: 1596-1603.
[52] Bloomgren G, Richman S, Hotermans C, Subramanyam M, Goelz S, Natarajan A, Lee S, Plavina T, Scanlon JV, Sandrock A, Bozic C. Risk of natalizumab-associated progressive multifocal leukoencephalopathy. N Engl J Med 2012; 366: 1870-1880.
[53] Warnke C, Adams O, Hartung HP, Gold R, Hemmer B, Hohlfeld R, Stangel M, Zipp F, Wiendl H, Kieseier BC. Risk stratification of progressive multifocal leukoencephalopathy under natalizumab: recommendations for JC virus serology. Nervenarzt 2011; 82: 1314-1319.
[54] Warnke C, Mausberg AK, Stettner M, Dehmel T, Nekrich L, Meyer zu Horste G, Hartung HP, Fogdell-Hahn A, Adams O, Kieseier BC. Natalizumab affects the T-cell receptor repertoire in patients with multiple sclerosis. Neurology 2013; 81: 1400-1408.
[55] Gaspar HB, Cooray S, Gilmour KC, Parsley KL, Adams S, Howe SJ, Al Ghonaium A, Bayford J, Brown L, Davies EG, Kinnon C, Thrasher AJ. Long-term persistence of a polyclonal T cell repertoire after gene therapy for X-linked severe combined immunodeficiency. Sci Transl Med 2010; 3: 97-79.
[56] Hacein-Bey-Abina S, Garrigue A, Wang GP, Soulier J, Lim A, Morillon E, Clappier E, Caccavelli L, Delabesse E, Beldjord K, Asnafi V, MacIntyre E, Dal Cortivo L, Radford I, Brousse N, Sigaux F, Moshous D, Hauer J, Borkhardt A, Belohradsky BH, Wintergerst U, Velez MC, Leiva L, Sorensen R, Wulffraat N, Blanche S, Bushman FD, Fischer A, Cavazzana-Calvo M. Insertional oncogenesis in 4 patients after

retrovirus-mediated gene therapy of SCID-X1. J Clin Invest 2008; 118: 3132-3142.
[57] Dziubianau M, Hecht J, Kuchenbecker L, Sattler A, Stervbo U, Rödelsperger C, Nickel P, Neumann AU, Robinson PN, Mundlos S, Volk HD, Thiel A, Reinke P, Babel N. TCR repertoire analysis by next generation sequencing allows complex differential diagnosis of T cell-related pathology. Am J Transplant 2013; 13: 2842-2854.
[58] Klarenbeek PL, Remmerswaal EB, ten Berge IJ, Doorenspleet ME, van Schaik BD, Esveldt RE, Koch SD, ten Brinke A, van Kampen AH, Bemelman FJ, Tak PP, Baas F, de Vries N, van Lier RA. Deep sequencing of antiviral T-cell responses to HCMV and EBV in humans reveals a stable repertoire that is maintained for many years. PLoS Pathog 2012; 8: e1002889.
[59] Appelbaum FR, Forman SJ, Negrin RS, et al. Thomas' Hematopoietic Cell Transplantation. West Sussex, UK: Blackwell Publishers; 2009.
[60] Storek J, Geddes M, Khan F, Huard B, Helg C, Chalandon Y, Passweg J, Roosnek E. Reconstitution of the immune system after hematopoietic stem cell transplantation in humans. Semin Immunopathol 2008; 30: 425-437.
[61] Seggewiss R, Einsele HH. Immune reconstitution after allogeneic transplantation and expanding options for immunomodulation: an update. Blood 2010; 115: 3861-3868.
[62] Maury S, Mary JY, Rabian C, Schwarzinger M, Toubert A, Scieux C, Carmagnat M, Esperou H, Ribaud P, Devergie A, Guardiola P, Vexiau P, Charron D, Gluckman E, Socié G. Prolonged immune deficiency following allogeneic stem cell transplantation: risk factors and complications in adult patients. Br J Haematol 2001; 115: 630-41.
[63] Nikolich-Zugich J, Slifka MK, Messaoudi I. The many important facets of T-cell repertoire diversity. Nat Rev Immunol 2004; 4: 123-132.
[64] Muraro PA, Robins H, Malhotra S, Howell M, Phippard D, Desmarais C, de Paula Alves Sousa A, Griffith LM, Lim N, Nash RA, Turka LA. T cell repertoire following autologous stem cell transplantation for multiple sclerosis. J Clin Invest 2014; 124: 1168-1172.
[65] van Heijst JW, Ceberio I, Lipuma LB, Samilo DW, Wasilewski GD, Gonzales AM, Nieves JL, van den Brink MR, Perales MA, Pamer EG. Quantitative assessment of T cell repertoire recovery after hematopoietic stem cell transplantation. Nat Med 2013; 19: 372-377.
[66] Lev A, Simon AJ, Bareket M, Bielorai B, Hutt D, Amariglio N, Rechavi G, Somech R. The Kinetics of Early T and B Cell Immune Recovery after Bone Marrow Transplantation in RAG-2-Deficient SCID Patients. PLoS One 2012; 7: e30494.
[67] Iqbal N, Salzman D, Lazenby AJ, Wilcox CM. Diagnosis of gastrointestinal graft-versus-host Disease. Am J Gastroenterol 2000; 95: 3034-3038.
[68] Koff WC. HIV vaccine development: challenges and opportunities towards solving the HIV vaccine-neutralizing antibody problem. Vaccine 2012; 30: 4310-4315.
[69] González N, Alvarez A, Alcamí J. Broadly neutralizing antibodies and their significance for HIV-1 vaccines. Curr HIV Res 2010; 8: 602-612.
[70] Xiao X, Chen W, Feng Y, et al. Germline-like predecessors of broadly neutralizing antibodies lack measurable binding to HIV-1 envelope glyco-proteins: implications for evasion of immune responses and design of vaccine immunogens. Biochem Biophys Res Commun 2009; 390: 404-409.
[71] Wu X, Zhou T, Zhu J, Zhang B, Georgiev I, Wang C, Chen X, Longo NS, Louder M, McKee K, O'Dell S, Perfetto S, Schmidt SD, Shi W, Wu L, Yang Y, Yang ZY, Yang Z, Zhang Z, Bonsignori M, Crump JA, Kapiga SH, Sam NE, Haynes BF, Simek M, Burton DR, Koff WC, Doria-Rose NA, Connors M; NISC Comparative Sequencing Program, Mullikin JC, Nabel GJ, Roederer M, Shapiro L, Kwong PD, Mascola JR. Focused evolution of HIV-1 neutralizing antibodies revealed by structures and deep sequencing. Science 2011; 333: 1593-1602.
[72] Larimore K, McCormick MW, Robins HS, Greenberg PD. Shaping of human germline IgH repertoires revealed by deep sequencing. J Immunol 2012; 189: 3221-3230.
[73] Li S, Lefranc MP, Miles JJ, Alamyar E, Giudicelli V, Duroux P, Freeman JD, Corbin VD, Scheerlinck JP, Frohman MA, Cameron PU, Plebanski M, Loveland B, Burrows SR, Papenfuss AT, Gowans EJ. IMGT/HighV QUEST paradigm for T cell receptor IMGT clonotype diversity and next generation repertoire immunoprofiling. Nat Commun 2013; 4: 2333.
[74] Thomas PG, Handel A, Doherty PC, La Gruta NL. Ecological analysis of antigen-specific CTL repertoires defines the relationship between naïve and immune T-cell populations. Proc Natl Acad Sci U S A 2013; 110: 1839-1844.
[75] Zhu J, Peng T, Johnston C, Phasouk K, Kask AS, Klock A, Jin L, Diem K, Koelle DM, Wald A, Robins H, Corey L. Immune surveillance by CD8aa+ skin-resident T cells in human herpes virus infection. Nature 2013; 497: 494-497.
[76] Becker RS, Knight KL. Somatic diversification of immunoglobulin heavy chain VDJ genes: evidence for somatic gene conversion in rabbits. Cell 1990; 63: 987-997.
[77] Beck A, Wurch T, Bailly C, Corvaia N. Strategies and challenges for the next generation of ther-

apeutic antibodies. Nat Rev Immunol 2010; 10: 345-352.
[78] Talapatra A, Rouse R, Hardiman G. Protein micro-arrays: challenges and promises. Pharmacogenomics 2002; 3: 527-536.
[79] Hoogenboom HR. Selecting and screening recombinant antibody libraries. Nat Biotechnol 2005; 23: 1105-1116.
[80] Lanzavecchia A, Corti D, Sallusto F. Human monoclonal antibodies by immortalization of memory B cells. Curr Opin Biotechnol 2007; 18: 523-528.
[81] Sullivan M, Kaur K, Pauli N, Wilson PC. Harnessing the immune system's arsenal: producing human monoclonal antibodies for therapeutics and investigating immune responses. F1000 Biol Rep 2011; 3: 17.
[82] Fischer N. Sequencing antibody repertoires: the next generation. MAbs 2011; 3: 17-20.
[83] Wu TT, Kabat EA. An analysis of the sequences of the variable regions of Bence Jones proteins and myeloma light chains and their implications for anti-body complementarity. J Exp Med 1970; 132: 211-250.
[84] Hardiman G. Application of ultra-high throughput sequencing and microarray technologies in pharma-cogenomics testing. In: Dasgupta A, editor. Therapeutic Drug Monitoring. London: Elsevier; 2012. pp. 144-159.
[85] Larman HB, Xu GJ, Pavlova NN, Pavlova NN, Elledge SJ. Construction of a rationally designed antibody platform for sequencing-assisted selection. Proc Natl Acad Sci U S A 2012; 109: 18523-18528.
[86] Weinstein JA, Jiang N, White RA, Fisher DS, Quake SR. High-throughput sequencing of the zebrafish antibody repertoire. Science 2009; 324: 807-810.
[87] Cheung WC, Beausoleil SA, Zhang X, Sato S, Schieferl SM, Wieler JS, Beaudet JG, Ramenani RK, Popova L, Comb MJ, Rush J, Polakiewicz RD. A proteomics approach for the identification and cloning of monoclonal antibodies from serum. Nat Biotechnol 2013; 30: 447-452.
[88] DeKosky BJ, Ippolito GC, Deschner RP, Lavinder JJ, Wine Y, Rawlings BM, Varadarajan N, Giesecke C, Dörner T, Andrews SF, Wilson PC, Hunicke-Smith SP, Willson CG, Ellington AD, Georgiou G. High-throughput sequencing of the paired human immunoglobulin heavy and light chain repertoire. Nat Biotechnol 2013; 31: 166-169.
[89] Robins HS, Campregher PV, Srivastava SK Wacher A, Turtle CJ, Kahsai O, Riddell SR, Warren EH, Carlson CS. Comprehensive assessment of T-cell receptor β-chain diversity in αβ T cells. Blood 2009; 114: 4099-4107.