TCR-clonotyping-based Analysis of a Frenemy - Public T Cells

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Abstract─ Public T cells are characterized by a T-cell population bearing identical T-cell receptor (TCR) amino acid sequences in a majority of individuals. However, due to their out-of-balance production, they may serve as both "friend" and "enemy", i.e. a frenemy in many physiological and pathological activities. Thanks to the development of advanced TCR-clonotyping methods, such as next generation sequencing (NGS), we can gain a better understanding of public T-cell responses. In this review, we commenced with the underlying mechanisms of public TCR-production, and then discussed some researches that have been successfully utilized as a new method for the study of the CDR3 regions of the public and private TCRs, and we further explored the merits and demerits of the public T cells in different diseases. Finally, we discussed the current challenges and future hot focuses.

Keywords─ *public T cells, TCR clonotyping, public TCR, private TCR, NGS*

I. INTRODUCTION

When a mature T-cell receptor (TCR) specially recognizes a peptide presented by self MHC molecule, adaptive immune responses are selectively activated. Although the need to assure tolerance acts as a selective counterbalance towards self antigens, there are plenty of potential self and foreign antigens recognized by an extremely diverse TCR repertoire during a lifelong time [1, 2]. The recognition specificity and binding affinity of a TCR, to a large extent, depend on the three complementarity-determining regions (CDR1-3). The first and second CDRs in the TCRs are encoded by the germline genes, which are much more conservative than the CDR3 regions. The formation of the CDR3 regions undergoes a complex series of gene rearrangement, which is generated within the thymus during T-lymphocyte development through the recombination of discrete V, (D), J gene segments in the TCR chains [3]. At CDR3 recombination junctions, further diversity is generated through the exonuclease trimming of germline-encoded nucleotides and the addition of random nontemplate-encoded nucleotides [4, 5]. So, the resulting hypervariable CDR3 region is a key determinant to antigenic specificity. That is why analysis of the CDR3 diversity is crucial for the understanding of the basic molecular mechanisms of adaptive immunity in change-tracking function in health and disease.

Although classical Sanger sequencing has made a great contribution to genomic sequencing, such technique for estimating TCR diversity appears to be time-consuming and costly, and it only can generate limited data [6]. Thanks to the development of single-cell and cytometry methodologies, and shallow and deep TCR clonotyping, now scientists will be able to use these methods to revolutionarily analyze the CDR3 regions of TCRs, thereby further speculating the whole TCR repertoire diversity [2, 3, 7]. Herein, peptide-MHC tetramers and flow cytometry are two common methods being widely used to directly detect and sort out the given antigen-specific T cells. As MHC class I molecules are easier to assemble and stabilize in vitro, the CD8+ T-cell populations are studied more than the CD4+ ones in TCR diversity analysis [8]. Moreover, due to too many unknowns and variabilities as to the targeted epitopes, it may cause the limitation to the mentioned methods above. Fortunately, another new methodology, the next-generation sequencing (NGS) ─ one deep-TCR-clonotyping method, has overcome the limitation by identifying and tracking TCR clonotypes, irrespective of whether the targeting antigen is known or unknown. Providing there is an immediate impact on the immune-repertoire diversity, NGS may have the potential to determine the TCR diversity, thereby revealing varieties of physiological and pathological characteristics. Due to lower background, higher sensitivity and resolution, NGS is widely being using at a blistering speed [7]. So to speak, NGS, a kind of "now-generation" sequencing, will be used as a widespread diagnostic, prognostic and predictive strategy sooner or later [3, 9].

Some studies have mainly focused on estimating the TCR repertoire diversity in certain diseases via the quantitative analysis of distinct TCR clonotypes within one individual [5, 10]. Despite NGS has created an unprecedented opportunity to implement high throughput analysis of the TCR and immunoglobulin repertoires of both animals and humans, the resulting data is still a drop in the bucket [11]. Determining the true size of TCR repertoire remains intractable. However, albeit the enormous repertoire, the immune responses often exhibit a biased TCR selection, leading to a population of "public" T cells dominating a relevant immune response against a specific antigen among the majority of individuals [12-14]. The public T cells are defined as those sharing identical TCR amino acid sequences among different individuals. Recently, growing attention has been paid to studying

the public T cells. Besides, there are all sorts of immune challenges encountered in our life-long period, such as malignancy, transplantation, infection, autoimmunity, and even ageing [4]. Under these conditions, without prior antigenic information, we can easily figure out a responsive TCR in common among individuals via the method of TCR-clonotyping analysis. However, public T cells have a complex function behaving as a frenemy: sometimes being a friend, but sometimes being a foe. In this review, we addressed the underlying mechanisms of public TCR-production. Moreover, we discussed some researches that have been successfully utilized as a new method for the study of the CDR3 regions of the public and private TCRs, and we further explored the merits and demerits of the public T cells in different diseases.

II. THE PRODUCTION OF PUBLIC TCR CLONOTYPES

For a public TCR, it is supposed to eventually survive in the naïve repertoire to perform similar functions among individuals [15]. That is, T-cell population bearing identical TCR must undergo a complex and rigorous process of selection and survival, such as the initial V, (D), J recombination, positive and negative selection within the thymus, and the peripheral survival. Besides, proper binding affinity to a given antigenic epitope is also a prerequisite for a TCR to be sharing [16]. Therefore, on a probabilistic basis, the shared TCRs seem to be rarely seen. However, on the contrary, public T cells never flinch from these difficulties. Multiple studies have shown the interindividual shared TCR repertoire is much larger than expected at random. Public T cells are even observed at high frequency in certain immune responses, such as ageing, human hepatitis C virus infection, HIV, chronic beryllium disease, and Rheumatoid Arthritis [10, 13, 15, 17-19].

Here comes the question "How can these repeatedly selected TCR sequences dominate these responses?" There are two underlying mechanisms contributing to the public TCR repertoire being widely accepted. One is the "recombinatorial biases", including V, (D), J usage biases and the biased number of inserted and deleted bases at the V, (D), J junctions [20]. Public CDR3 sequences tend to be closer to germline. In other words, CDR3 sequences with fewer additions and deletions are generated at a high frequency [21]. Such preferential recombination makes it easier to generate a population of shared TCR sequences among individuals, indicating it is a frequent nonrandom process [22]. The other one is the "convergent recombination", which is of great importance to shape the naïve TCR repertoire [20]. Due to the gene-code degeneracy, the identical CDR3 amino acid (aa) sequences could be translated from different nucleotide recombinations [5, 23]. In terms of probability, it seems that public CDR3 aa types would enjoy a more selective advantage than nucleotide types [24]. Thus, the public T-cell responses may lead to the preservation of certain preferred aa types obtained from a larger

diversity of specific nucleotide sequences [25]. Maryam yassai et al [22] demonstrated that it is common in HLA-A2 individuals that BV19-expressing CD8 T-cell responses often accompany with the utility of an 11-aa-long CDR3 with Arg and Ser (RS) expressing at the 5, 6 residue position respectively, showing evidence that biased recombination may favor particular positions in the CDR3 regions.

In addition to the two accepted mechanisms mentioned above, some studies [26, 27] suggested that thymic selection also has an inextricable link with the further generation of publicness. It has been suspected that highly restricted public T cells may be selected via recognizing the bulged peptide epitopes, while "featureless" epitopes with fewer exposed residues for TCR interaction may select a diverse TCR repertoire [26]. But this hypothesis still remains debatable as the opposite result of the function of featured vs. featureless epitopes has been reported [27]. Moreover, Geogina Thorborn et al [14] deduced that allelic polymorphisms may also contribute to a biased recombination, leading to generate the optimal public epitope-specific clonotypes. In this context, the complexity of the production patterns of public T cells is beyond our imagination and has yet to be resolved.

III. PUBLIC-PRIVATE PARTNERSHIP

Referring to the public T cells, we have to mention the private T cells as well. Public T cells are found among the bulk of individuals, while the majority of distinct antigen-specific TCR clonotypes within one individual are private that may not be found in others, i.e. private TCRs [8]. The antigen-specific responses, however, appear to include both the public and private specificities, no matter in diverse or highly-skewed antigen-specific TCR repertoire. In other words, in most cases, the public responses do not exist independently, but rather exist with some private specificities [28]. TCR sequences can range from private to highly public. Interestingly, both private and public TCRs may likely have the very frequent sequences that play a predominant role in a specific response. Thus, the difference between public and private TCRs is not due to their relative frequency, but due to the extent of convergent recombination [24].

One study showing analysis results of overall repertoire overlap between T-large granular lymphocyte (T-LGL) leukemia patients and healthy controls suggests that T-LGL leukemia clonotypes are private to the disease at both the individual level and the global T-LGL leukemia disease level [29]. Remarkably, private TCR repertoires may have the potential to increase the diversity of any given population and enhance the overall fitness [30]. We can create a bold speculation that these individual-specific T cells may provide a very good breakthrough for drug development by changing their individualities into commonnesses and eventually benefit the whole patients suffering from the same disease [31].

IV. THE MERITS AND DEMERITS OF PUBLIC T CELLS IN DIFFERENT IMMUNE CHALLENGES

Although public T cells are widespread in many physiological and pathological activities, they have merits as well as demerits. Normally, public TCR-recognition of foreign antigens is essential for immune defense during some infections and anti-tumor responses. Whereas some self-reactive public T cells, which recognize self-peptides and escape thymus selection, may be a potential trigger of autoimmune disease or retain their ability of self-recognition in transplantation. Worse still, the out-of-balance production of public T cells may also lead to the dysfunction of the immune homeostasis, thereby posing a health hazard. In the following sections, we discussed the different roles of public T cells in different immune challenges: friendship or hostility?

A. Tumor-related Public T Cells

Tumor-specific antigens within a limited environment may cause the oligoclonal expansion of antigen-specific T cells, yeilding a restricted and specific TCR repertoire [23]. And many tumor-specific TCR clonotypes seem more likely to originate from the predictable public and private clonotypes, among which public responsive T regulatory cell (Treg) plays an indispensable role [14, 25]. These Tregs may be generated within thymus and then migrate to tumor areas to expand and accumulate. Alexander Sainz-Perez et al [32] speculated a rare Teff-Treg conversion process that the exhaustion or clonal deletion of intratumoral effector T cells (Teff) may make it easier for the expansion of intratumoral public Tregs to access antigens and shape clonal dominance. Hopefully, public Treg-mediated tumor-suppressor responses may serve either as an effective mediator for target-specific immunosuppression, or as a prognostic marker to predict clinical treatment effect at one day.

In addition, a study [33] has shown that public and private T cells have comparable advantages in recognizing tumor cells, so to gain a better understanding of both public and private dominant tumor-related TCR clonotypes is a crux to effectively recognize tumor cells and further optimize the relevant therapy regimens. Furthermore, it will be beneficial if we can develop a public/private tumor-reactive TCR library among different individuals with different HLA alleles [34].

B. Transplantation-associated Public T Cells

Some kinds of allograft rejection start with public tissue-specific T-cell responses, which target the relevant allopeptides in the transplantation recipients. Applying a blockade on these T cells will contribute to a successful transplantation [35]. However, from another point of view, such public T cells may well provide a new understanding as to transplant rejection at the molecular level. We can focus attention on tracking their presence, enrichment, and activities during the posttransplant period in order to monitor the influence of posttransplant immunity [36]. Thus, the selected public T-cell response will be a target for inducing the donor-specific transplantation tolerance.

As for transplant rejection, it seems there exist some cross-talk responses dominated by the shared TCRs as well. M.Dziubianau et al [37] tried complex diagnosis of T-related pathology by analyzing the clonotype overlap between different specimens in clinical settings, including BKV- (polyomavirus BK), alloantigen-specific T cells, kidney allograft- and urine-derived lymphocytes. Such shared clonotypes among these clinical settings can be used to reveal the link between virus-specific T-cell clonotypes and post-graft function. And then, the given hints may help to adjust the appropriate measures to clear the virus and maintain the graft function. In this case, the overlap analysis may be of a significant clinical value in surveillance and diagnosis of allograft dysfunction and aid in the improvement of therapeutics.

C. Virus-specific Public T Cells

Sometimes, a response with high frequency of public T cells is not necessary as a good thing. Although the public antigen-specific T-cell responses observed early in the infection are speculated to perform first line of defense [38], too many public T-cell clonotypes could be a bad sign for "immunologic power" [9] and even reduce the resistance to diseases. The major reason is that a greater clonal breadth of public T cells may limit the overall magnitude of virus-specific responses [39], leading to a narrow TCR repertoire diversity. In addition, the limited immune function may fail to response rapidly to the emerging antigens or some hyper mutated RNA viruses, such as HIV, raising the risk of infection. For example, $L_{268}M$ mutation enables early-stage HIV to escape after public TRBV4-3/TRBJ1-3 clonotypes expand to replace the initial mobilized repertoire, although these clonotypes with high levels of antigen sensitivity may ensure effective HIV-suppressive activity [15]. It is uncertain to what extent should these public antigen-specific T cells engage in virus clearance or virus persistence [19]. Maybe there exists a threshold to assess the balance of benefit and harm [31]. Padma Billam et al [28] have mentioned at this point that sometimes it is the subdominant yet highly-functional cytotoxic lymphocyte (CTL) responses, rather than the dominant ones, that can effectively control viral replication, regardless of their public or private specificities. Thus, how to control the cutoff point towards the beneficial development and avoid virus escape is worth deeply analyzing, which is an important concern for vaccine design.

D. Autoimmunity-related Public T Cells

There also seems to be a relationship between some public T cells and autoimmunity. High frequency of self-related public T cells can be explained by the fact that T-lymphocytes will encounter plenty of self

antigens during their development within thymus, leading to a high degree of convergent recombination [24]. These public T cells may have a low affinity to self antigens so that they can survive from thymic selection. Normally, they may play a vital role in controlling autoimmunity as well as maintaining immune tolerance. However, if they are out of balance in production, it may be a causative factor for induction of autoimmunity, posing a threat to health instead. Surprisingly, some ethnic groups with particular HLA alleles have taken some countermeasures to avoid using certain public T cells through clonal deletion during thymocyte negative selection, because these public T cells may be alloreactive with their HLA alleles [12]. On the upside, we can analyze the autoimmunity-related public T cells through TCR-clonotyping methods and then characterize the self antigens they recognize, thereby enhancing our knowledge of the underlying triggers and drivers of autoimmunity.

E. Age-related Public T Cells

Age-related immune decline appears to be connected with the changes in TCR publicness. One study showed that the reduced diversity of splenic T-cell repertoire in aged mice is because a private set of TCR clonotypes gradually expands to a dominance in the repertoire [40]. Distinct and private repertoires will likely occupy the immunological niche in a stepwise fashion among old individuals. A similar study on aged people also displayed that the number of representative public TCR clonotypes declines with age and shows a pattern similar to that of naive T cells, suggesting the majority of these high-frequency public clonotypes are naive T cells [10]. Judging from that, the underlying relationship between young and ageing public T cells really has to do something with the thymus involution and may help to reveal the influence of ageing on adaptive immune system.

V. CHALLENGE AND FUTURE

However, we are not sure whether these experimentally obtained public T cells are true or not. Since current data remains unreliable and still has some loopholes, future work should center on the improvement of technologies and methodologies.

First, the selection for public T cells is a TCRα- and β-chain-dependent process and both chains may play an equivalent role in recognizing peptide specificity [41]. However, most studies have mainly focused on the TCRβ-repertoire sequencing, omitting half of the truth. In addition, due to the biased amplification process in NGS, the results may tend to extremes that lead to overexpress or underexpress the sequences of interest [42]. Therefore, more attentions should be paid to engaging in linking the two specific chains of a receptor and accomplishing the single-cell sequencing without amplification. It is likely to have a reverse effect that after improving the detection reliability and pairing with the relevant α chains, private TCR β

sequences may tend to be public ones with low frequency and a public TCR β chain may otherwise turn to be a private TCR αβ heterodimer [8, 28, 43].

Second, the avidity-based TCR-peptide-MHC interaction and the relevant structural constraints may be key determinants of public T-cell selection, in that biased clonotypes with high-avidity TCRs may, at least in part, reflect an optimization of signaling [41]. Therefore, it is impossible to solely rely on sequence information, which may be mapped much less onto the three-dimensional conformation of the receptors [44]. Thus, predicting the three-dimensional interaction structure of TCR-peptide-MHC complex should take priority so as to further analyze the relationship among the antigen specificity, the MHC anchoring site and the degree of affinity interaction（high or low）, and unlock some hidden information.

Besides the above, different pairings of TCR β chains and distinct TCR α chains may create different levels of affinity of public TCRs and then result in different functional outcomes, leading to the changes in cytokine production. In this circumstance, in-vitro studies such as comprehensive serology assessment should be applied to offer supplementary information and assist to distinguish between the "best binding" and "just adequate" interactions [29, 45], providing insight into the development of relevant drugs and/or vaccines.

VI. CONCLUDING REMARKS

Current researches have made great progress in the studies of public T-cell responses, but the restrained technologies and methodologies may be a major block for further exploration of their real identity. We still don't know whether the public T cell is the cause or the result in the involved physiological and pathological changes. Thus, it will be necessary and beneficial to probe the real role of public T cells in different responses. Moreover, a better understanding of the public T-cell responses can enable us to fully take advantage of their merits while avoid their demerits. Hopefully, it is only a matter of time until the public specificity among individuals would help to uncover more daunting immune challenges with the TCR-clonotyping method.

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