SHORT ANALYTICAL REVIEW

Re-shaping the T cell repertoire: TCR editing and TCR revision for good and for bad

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Abstract Protection against the universe of pathogens requires a functional, diverse T cell repertoire. However, the price that is paid for an evolved, effective immune system includes the potential danger of generating autoaggressive T cells. Autoimmune diseases result from inherent breach of tolerance to self-antigens leading to disruption of the regulatory to autoaggressive T cell homeostatic balance. The immune system has evolved mechanisms to control those processes. For T cells, positive and negative selection in the thymus assures that only fully functional, non-self-reactive T cells will populate the periphery. Failure of this central tolerance would result in autoaggressive T cells escaping into the periphery. However, other means of escaping negative selection can occur in the periphery, i.e., TCR revision, or the altering of TCR expression after thymic egress. Here the potential benefits, i.e., expansion and re-shaping of the T cell repertoire as potentiated by TCR editing and revision are considered. Furthermore, the potential to develop autoaggressive TCR and thus enhance autoimmunity is considered.

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Maintaining immunity to a universe of pathogens requires intricate cooperation between the innate and adaptive immune systems, which collectively are responsible for providing protection. Each branch has evolved highly specialized and essential functions to provide both immediate and long-term protection. The innate immune arm includes macrophages and neutrophils each responsible for removal of pathogens by direct physical attack, thereby making it adept at immediate immunity. For example, a macrophage phagocytoses a pathogen becoming activated to produce pro-inflammatory cytokines like IL-1β [1], TNFα [2] and reactive oxygen intermediates that destroy bacteria [3]. The adaptive arm is composed of B and T lymphocytes and is certainly responsible for removal of pathogens but has evolved to provide long-term immunity or memory B and T cells.

Development of adaptive immunity

The adaptive immune system is dependent upon the ability of T and B cells to diversify during their development. Protective immunity requires an expanded repertoire of T and B cells, capable of recognizing and responding to pathogens through expression of an antigen specific receptor. Each T cell and B cell typically exhibits restricted antigen specificity, therefore a panoply of different lymphocytes each carrying a unique antigen specificity must develop and be maintained. T and B lymphocytes undergo similar development stages acquiring their respective receptors, T cells (TCR) in the thymus and B cells (BCR) in bone marrow.

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Progenitor T cells described as double negative, i.e., lacking expression of CD4 and CD8, the molecules that will ultimately define the functional status of T cells, are generated in the bone marrow and migrate to the thymus. A signal of yet unknown origin initiates synthesis of RAG1 and RAG2, the TCR recombinase proteins. The RAG1/RAG2 complex binds to the TCR\(\beta\) gene through recognition of specific, highly conserved DNA sequences described as recombination signal sequences. The RAG complexes loop out segments of DNA, and the recombinase activity splices the DNA. The affected DNA is transcribed into messenger RNA that codes for a specific TCR\(\beta\) chain. The TCR\(\beta\) gene experiences allelic exclusion, that is, when rearrangement occurs on one TCR\(\beta\) allele, the other allele is excluded from rearrangement. The beta gene is composed of variable (V), diversity (D) and joining (J) segments. The order of rearrangement, mediated by the RAG1/RAG2 complex is D to J, then V to DJ. The gene loci exist in translocons which are groupings of Vs, Ds, and Js that associate with one of two constant regions. Recent studies indicate that, rather than complete allelic exclusion, the TCR\(\beta\) genes undergo intra-allelic ordering, which ensures that V\(\beta\) to DJ\(\beta\) is the final step of recombination and results in feedback preventing further rearrangements. Rather than the opposite beta allele being completely excluded, the D\(\beta\) to J\(\beta\) recombination occurs but the final V\(\beta\) to DJ\(\beta\) joining on the second translocon only happens if the original recombination is unsuccessful. The final V(D)J segment is tested for in-frame alignment. This process is relatively random, so that in one particular developing T cell, a TRBV2 transcript is generated but in a neighboring cell a TRBV8 transcript may result. The newly synthesized TCR\(\beta\) protein associates with a pre-TCR\(\alpha\), and this pre-TCR is expressed. At this point during development, RAG1 and RAG2 become silenced, which may involve interaction of the pre-TCR with MHC/antigen. In fact, expression of the pre-TCR is required for T cells to progress to the double positive, CD4\(^+\)CD8\(^+\), stage of development suggesting that selective interactions involving the pre-TCR are critical for development.

Once developing T cells reach the CD4/CD8 double positive stage, the RAG1/RAG2 complex again is induced. IL-7 signals open the chromatin making the DNA accessible, but the actual RAG1/RAG2 induction signal is unknown. Re-induction of RAGs at the double positive development stage leads to TCR\(\alpha\) gene recombination. The process is the same however the alpha gene is composed of V and J regions only. More than one TCR\(\alpha\) chain can be expressed. The pairing of TCR\(\beta\) and TCR\(\alpha\) provides antigen specificity to T cells. The fact that these processes happen in millions of different cells provides the diversity that is required of the immune system.

**Positive and negative selection**

Once the TCR protein is expressed, a critical juncture in the development of T cells occurs. Because the actions of RAG1/RAG2 are somewhat random, the newly generated TCR repertoire could include T cells that are not responsive to the host’s MHC complexes. MHC molecules are polymorphic, and therefore variation in MHC expression occurs among individuals. T cells are MHC restricted and MHC/antigen interactions are vital to provide the survival signal for developing T cells. Only T cells carrying TCR molecules that

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**Figure 1** Cartoon representing RAG1 and RAG2 mediated TCR recombination on the \(\beta\) chain gene. The TCR genes are organized into Variable (V), Diversity (D) and Joining (J) regions and a constant (C) region. The current theory is that one of two constant regions is joined to a series of V, D and J to comprise translocons. RAG1 and RAG2 form a complex that binds to the D and J regions of one of the translocons, splicing out the interval DNA to create a DJ join. The next round involves the RAG complex binding to a V region that then is connected to the DJ region creating a V(D)J join. The DNA then is transcribed to mRNA, and the protein is translated. This process is controlled but the outcome is relatively random.
can recognize the host’s available MHC are positively selected to survive by receiving signals through the TCR. Developing T cells that cannot recognize the available MHC complexes undergo death by neglect. For example the H-Y TCR transgenic mouse was constructed with T cells that respond to a Y chromosome antigen. H-Y mice carry H-2D^b [14]. Another TCR transgenic mouse, OT-I has T cells that recognize a portion of the ovalbumin peptide and are restricted to H-2K^b [15]. A demonstration of positive selection would be if OT-I T cells are exposed to H-2D^b, they are not restricted to that MHC, therefore cannot respond and die by neglect (Fig. 2A).

The next critical phase in T cell development is removal of those T cells that can respond to self-antigen and therefore pose the danger of auto-reactivity. The mechanism to prevent this danger is negative selection or central tolerance. Early studies using TCR transgenic mice predicted that the necessary fate for any T cell carrying a self-antigen high affinity TCR was induced cell death. Experimentally, this was shown in the H-Y TCR transgenic mouse where male transgenic mice do not develop T cells but female mice do [14]. Because male mice express the Y self-antigen, when T cells encounter that antigen presented by H-2Db, the T cells deleted [14], shown in Fig. 2B. In female H-Y TCR-Tg mice, T cells develop normally because even though female H-Y TCR-Tg mice carry the H-2D^k restricted T cells no Y self-antigen is available. The conclusion was that negative selection resulted in cell death. Another twist to that story is that, when H-Y mice are on an H-2D^b background, which was shown capable of presenting the H-Y antigen, deletion does not occur, but rather developmental arrest occurs [16]. These data would suggest a role for MHC presentation independent of antigen strength in deciding the fate of the T cell (Fig. 2C).

TCR editing and negative selection

The concept of negative selection continues to evolve however. The idea that developing T cells are capable of undergoing TCR editing after a failed attempt at negative selection has emerged. The description of receptor editing originated in B cells [17—20]. In that model, it was shown that B cells can edit the B cell receptor, effectively changing antigen specificity, during development in the bone marrow [17,18,21]. T cells also undergo receptor editing. The OT-I, TCR-Tg, mouse has T cells that recognize ovalbumin (OVA) peptide sequence 257—264 and carry an H-2K^b haplotype [22]. Mice were generated that express OVA, as a neo-self-antigen, in the medulla or in the thymic cortex. Positive

Figure 2 Positive and negative selection and TCR editing in the thymus. (A) T cells encounter MHC and antigen in the thymus. If the newly generated TCR can recognize the available MHC, it is positively selected to survive. If the TCR does not recognize the available MHC such as OT-I and H-2D^b, the cell undergoes death by neglect. (B) Some TCR molecules recognize a high affinity self-antigen such as H-Y TCR seeing the Y chromosome antigen presented by H-2Db and undergo induced cell death, negative selection/central tolerance. (C) In the case of the H-Y system, the H-Y TCR can recognize Y chromosome antigen presented by H-2A^b, the T cells do not undergo cell death, but rather are induced to clonal arrest. (D) Other T cells such as OT-I that recognize a force expressed, neo-self-antigen, presented by H-2K^b undergo TCR editing.
selection occurs in the thymic cortex, while negative is believed to occur in the medulla of the thymus [23]. These constructed OVA expressing mice were bred to OT-I mice. Rather than being deleted, a substantial portion of T cells remained intact. The interesting difference was that these surviving T cells no longer carried the transgenic TCR, but had undergone TCR editing to carry endogenous TCR molecules [22], which is represented in Fig. 2D. Further studies confirmed activation of the RAG1/RAG2 recombination complex and subsequent changes in TCR expression [24].

Revisiting the H-Y story, directly comparing deletion prone H-Y T cells to editing prone OT-I T cells, it was shown that H-Y TCR transgenic mice preferentially delete high affinity TCR bearing T cells when exposed to recognizable antigen while OT-I do not [22]. When the H-Y self-antigen was force expressed on the thymic cortical epithelial cells of female transgenic mice, the T cells deleted [22]. The conclusion from these data was that the decision to edit or delete was intrinsic to the T cell.

TCR revision and the T cell repertoire

While TCR editing in the strictest sense is confined to the thymus, this is not the only means by which T cells alter TCR expression. Immunology dogma has decreed that once T cells leave the thymus any further rearrangements of the TCR should not happen. However, studies now show that RAG1 and RAG2 can be induced in peripheral T cells and that, subsequent to that, alteration in TCR expression occurs [10,25–29]. This observation was first described using a TCR V\textsubscript{5} transgenic mouse, it was shown that RAG1/ RAG2 are induced in peripheral T cells and that, as the transgenic mice age, continued loss of V\textsubscript{5} expression on peripheral T cells was seen [30]. Even when thymectomized, V\textsubscript{5} + T cells continued to accumulate in the periphery [30]. Furthermore, TCR revision occurs preferentially in germinal centers [28], which arise in spleen and lymph nodes after antigen exposure. Germinal centers are the site for B cell affinity maturation and T–B cell interactions. In fact, TCR revision is dependent upon the presence of B cells [28,30], suggesting direct interaction between the revising T cell and an APC. In a different approach, it was reported that TCR revision can be driven by superantigens [29]. T cells respond to APC presented antigens and in fact are MHC restricted. Superantigens bind TCR directly to MHC non-specifically. During this interaction, T cells become activated and become tolerized [29,31]. One demonstrated feature of superantigen induced tolerance includes TCR revision [29].

An advantage of TCR revision is expansion of the T cell repertoire without the requirement for additional thymocyte production. Because mammals undergo thymic involution, suggesting a severe decrease in production of T cells, TCR revision would provide a means to maintain functional T cells. Because antigen specificity is directly linked to TCR expression, T cell specificity must necessarily change following induced TCR revision. This mechanism would prove highly conservational and practical. There is however a danger potential to these actions, i.e., the potential to generate autoaggressive TCR.

TCR revision and autoimmunity

While markers for autoaggressive T cells have proven elusive, a unique T cell effector subset described as CD4\textsuperscript{a}CD40\textsuperscript{b} was shown to be highly diabetogenic in the NOD mouse, type 1 diabetes model [32–34]. CD4\textsuperscript{a}CD40\textsuperscript{b} T cells initially are detected at expanded levels in pancreatic lymph nodes of NOD mice, while in the spleen and peripheral lymph nodes, CD4\textsuperscript{a}CD40\textsuperscript{b} T cells expand concurrently with insulitis, which is defined as infiltration of lymphocytes into the pancreatic islets [32]. Adoptive transfers of CD4\textsuperscript{a}CD40\textsuperscript{b} but not CD4\textsuperscript{a}CD40\textsuperscript{b} T cells from diabetic and, importantly, pre-diabetic (defined as low insulin and no hyperglycemia) NOD mice to NOD.scid recipients’ fulminate type 1 diabetes [32–34]. Little is known about CD40 signaling in T cells, but CD40 engagement of this T cell subset induced RAG1 and RAG2 expression [33]. Following RAG induction, TCR revision as demonstrated by alteration in \textit{V}\textsubscript{\alpha} expression in primary T cells and T cell clones was detected [32]. For example, early in development (3 weeks of age), NOD mice have low levels of TRAV2, TRAV3.2 and TRAV8.1.2 T cells [32]. When those T cells were CD40 engaged, the levels of TRAV3.2 T cells expanded significantly (from <1% up to 12% of total V\textsubscript{\alpha} T cells). The expanded levels were not due to induced proliferation or cell death [32]. At diabetes onset, NOD mice intrinsically carry elevated (14% of total V\textsubscript{\alpha} T cells) levels of TRAV3.2 T cells. A very interesting finding was that adoptive transfer of CD40 induced, TCR revised T cells proved highly diabetogenic [32]. These data support a mechanism for altering TCR expression in peripheral T cells that occurs under autoimmune conditions, and those revised T cells prove to carry high danger potential.

Both CD4\textsuperscript{a} and CD8\textsuperscript{b} T cells have been shown to be pathogenic in various autoimmune models. For example, a CD8\textsuperscript{b} T cell clone and subsequent TCR transgenic mouse have proven highly diabetogenic [35–37]. In marked contrast to TCR revision leading to autoaggressive T cell development, exposure of the diabetogenic CD8\textsuperscript{b} T cell clone to APC loaded with islet antigens induced RAG1 and RAG2 and subsequent changes in TCR expression [37]. This suggests that TCR revision can be T cell tolerogenic. Supporting this, we have seen that CD40 engagement of highly diabetogenic CD4\textsuperscript{a} T cell clones including BDC2.5 and BDC6.9 induces RAG1 and RAG2 expression, TCR revision and importantly loss of diabetogenicity in disease transfer experiments ([33] and laboratory observations).

Summary

TCR revision being both ‘dangerous’ and tolerogenic is not a mutually exclusive proposition. Developing T cells are capable of multiple alterations in TCR \textit{V}\textsubscript{\alpha} expression [38], and it has been shown that peripheral T cells can be induced to alter TRAV expression [32,39]. A model for T cell repertoire expansion that includes generation of autoaggressive T cells could be explained as follows. When the TCR of a peripheral T cell cannot recognize a presented antigen, one consequence is death by neglect (Fig. 3A). If such T cells can be induced to undergo TCR revision, several new opportunities both good and bad are now
available. First, revised T cells now may be able to respond to presented antigen, constituting expansion of the repertoire (Fig. 3B). A second outcome is that the newly revised TCR may be able to respond to a different presented antigen (Fig. 3B). This outcome further expands the repertoire. Some of the T cells, those that are responsive to presented antigen, could be shunted into memory phenotype and many of the responsive T cells will undergo activation induced cell death (AICD) [40]. Once antigens are cleared, the remaining T cells and those that are completely non-responsive to antigens will undergo death by neglect.

The final consequence would be generation of self-antigen responsive T cells and potential autoimmunity (Fig. 3C). Autoaggressive T cells even in non-autoimmune individuals do occur. However, autoimmunity only develops when T cells break tolerance. The first required step remains the ability to respond to a self-antigen, e.g. pancreatic antigens during type 1 diabetes. Tolerogenic mechanisms include induced cell death, anergy or regulatory T cell control of the autoaggressive TCR bearing T cells. Violation of one of these tolerogenic mechanisms leads to autoimmunity.

The development and evolution of the immune system are designed to protect us from pathogens be they external, virus, bacteria, etc. or internal, transformed cells. Survival depends upon an appropriately extensive T cell repertoire. TCR editing and TCR revision provide mechanisms to expand the repertoire and do so involving cellular conservation. However, the risk for developing autoaggressive T cells also exists. Ironically TCR revision may also prove protective by altering autoaggressive TCR molecules to no longer be a source of danger. Critical cellular tolerance mechanisms must maintain homeostasis of T cells, preventing autoimmunity.

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