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Memory T Cell-Specific Therapeutics in Organ Transplantation

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Abstract

Purpose of the Review—This review details the role of memory T cells in physiologic and allospecific immunity, and summarizes the effects of immunosuppressive agents used to manipulate their function in the context of organ transplantation.

Recent Findings—Memory T cells are lymphocytes with characteristics that are thought to promote anamnestic immune responses. They have a unique capacity to generate rapid effector functions upon secondary exposure to a pathogen, and this is achieved through truncated requirements for antigen presentation, reduced activation thresholds, and enhanced trafficking and adhesion mechanisms. In general, these same mechanisms also appear to evoke improved efficiency in mediating allograft rejection. The phenotype of these cells has been increasingly well defined and associated with a characteristic pattern of susceptibility to immunosuppressive agents. This knowledge is now being exploited in the development of immune therapeutic regimens to selectively mollify T memory cell effects.

Summary—A specific targeting of memory T cells has potential to prevent allograft rejection in a more precise manner than current means of immunosuppression. However, these benefits will be balanced by the reciprocal risk of susceptibility to recurrent infection.

Keywords

memory T cell; heterologous immunity; homeostatic proliferation; tolerance; allograft

Introduction

Immunological memory, or the ability to generate increasingly efficient antigen-specific protective immune responses with subsequent antigenic exposures, is a fundamental hallmark of adaptive immunity in higher vertebrates. The effect of an initial exposure to an environmental antigen is imprinted on a host organism's immune cell repertoire in such a way so as to increase the magnitude and rapidity of antigen clearance following re-exposure to that antigen. In particular, antigen-experienced T cells take on characteristics indicative of prior activation and give rise to a population of cells collectively referred to as memory T cells (T_M s). These cells mediate enhanced protection against invading pathogens and are thought to convey an evolutionary survival advantage. However, in the context of transplantation, the presence of cells with prejudiced reactivity against donor antigens increases the likelihood of immune mediated rejection such that adaptive immunity becomes counter-adaptive. While the precise pathways and cellular interactions that shape T_M function remain to be fully

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elucidated, emerging evidence suggests that these cells play a critical role in rejection. In this review we describe fundamental characteristics of T_{MS} , discuss their role in allograft rejection, and relate their unique traits to existing and emerging immune therapeutic agents.

Characteristics of Memory T Cells

T cells emerge from the thymus with a naïve or non-activated phenotype characterized by relatively high T cell receptor (TCR) density and limited adhesion molecule expression. This phenotype persists until the cell becomes primed. Priming requires repetitive binding of a cell's TCRs to major histocompatibility complex (MHC) molecules presenting the T cell's cognate peptide antigen in the context of sufficient costimulatory signals, accessory molecules and adhesion molecules to induce cell division. Following several rounds of division, naïve T cells differentiate into an activated, effector T cell population that then mediates antigen elimination. Most of these cells undergo apoptosis in the conduct of their effector function, leading to population contraction with antigen elimination. However, some cells persist as a pool of long-lasting antigen-specific T_{MS} . Two models have been suggested to describe the generation of T_{MS} from naïve precursors: a linear progression model postulating that memory populations arise from a pool of previously primed effectors, and a parallel progression model stipulating that memory populations develop as a separate lineage alongside the population of short-lived effectors [1–3]. In addition, recent evidence suggests that the development of T_{MS} may be influenced by antigen-specific T cell precursor frequency, the extent of antigenic stimulation, and/or the cytokine milieu present at the time of priming [4–6]. Antigen-specific T cell memory is maintained within the host by a basal homeostatic turnover that is thought to be supported independent of antigen by cytokines including IL-15 [7–11].

As compared to naïve T cells, T_{MS} possess distinct phenotypic, functional, and homing properties (Figure 1) [9,10]. They produce cytokines faster than naïve T cells, potentially from decreased activation thresholds [12], and possess direct cytolytic function *in vivo* following reencounter with antigen [13,14]. They also express higher levels of CD2, CD11a, and CD44 compared with their naïve counterparts and in humans express the RO isoform of CD45 as opposed to the RA isoform [10,15–18]. Numerous groups have demonstrated that altered expression of selectins, integrins, and chemokine receptors on T_{MS} are likely responsible for their unique homing properties including residence in peripheral tissues, allowing them more immediate access to peripheral antigen including alloantigen following transplantation [19–27]. While T_{MS} are heterogeneous, two well-described subsets exist within most antigen-specific memory populations. Central memory T cells (T_{CM} ; CCR7⁺ CD62L^{hi}) migrate primarily to secondary lymphoid tissues (e.g. lymph node and spleen) and are responsible for generating a burst of new effectors following recall. Effector-memory T cells (T_{EM} ; CCR7⁻ CD62L^{lo}) migrate to non-lymphoid tissues and provide immediate effector function at peripheral sites [19,26,27]. Whether these two populations derive from one another or have distinct origins is unclear, and there is evidence to support both paradigms [4,28,29].

In unsensitized transplant recipients, two unique mechanisms for the generation of donor-reactive T_{MS} have been described. First, heterologous immunity is the phenomenon whereby previous exposures to environmental pathogens influence the course of future immune responses to seemingly unrelated antigens [30–32]. Once thought to be exquisitely specific for a single peptide:MHC complex, TCRs are now appreciated to possess inherent degeneracy with regard to their recognition of antigen, such that a T cell recognizing one antigen can also respond to other antigens, albeit with altered affinity. Heterologous alloimmunity thus results when a T_M population primed by self-MHC presenting an environmental antigen generates cross-reactive T_{MS} responsive to allo- or self-MHC presenting an allopeptide. Recently, dual receptor T cells have been described as being over-represented in alloreactive T cell populations, raising the possibility that if a dual receptor T cell is activated via one pathogen-

specific TCR, it could later respond as a T_M if its second, alloreactive TCR were to encounter donor antigen [33].

Donor-reactive T_M s can also be generated via homeostatic proliferation, a process whereby transient lymphopenia caused by viral infection, or in the case of transplantation, therapeutic T cell depletion, induces the proliferation and differentiation of naïve T cells into cells with true phenotypic and functional characteristics of T_M s as well as cells that appear to be T_M s but fail to have robust effector functions [34–36]. Therefore, a proportion of the naïve alloreactive T cell pool is likely stochastically converted into alloreactive T_M s throughout life, giving most patients some degree of allo-responsive memory, although they have not had exposure to alloantigen.

Role of Memory T cells in Allograft Rejection

A growing body of evidence exists to suggest that T_M s may play a critical role in inhibiting allograft acceptance. For example, T_M s have recently been shown to have the ability to migrate into allograft tissues and secrete inflammatory cytokines well before the 4–6 days required for donor-specific priming in the spleen [27]. Furthermore, tolerance achieved in cardiac allografts using anti-CD154 are rejected when alloreactive T_M s are generated in recipients via sensitization with donor-type skin grafts [39,40]. In addition, regimens to induce tolerance have been highly successful in rodents, but less so in non-human primates (NHPs). One possible explanation for this is that longer lived, socially housed animals such as NHPs have, in general, greater exposure to environmental pathogens as compared to rodents housed in specific-pathogen free facilities, and therefore possess an increased opportunity to generate a diverse repertoire of T_M s [37,38]. Evidence supporting this hypothesis includes experiments in rodents where tolerance was achievable in naïve animals treated with CTLA4-Ig, anti-CD40L mAb, busulfan and donor bone marrow, but not in animals infected with LCMV, vaccinia virus, and vesicular stomatitis virus. Even after anti-viral immune responses were allowed to resolve to memory, the mice resisted tolerance induction. Stimulation of splenocytes isolated with these recipients with donor tissue resulted in increased frequency of cytokine producing cells, thus implicating the virus-elicited allo-crossreactive T_M s in the prevention of tolerance induction in this model [41].

Similar inhibition of tolerance has been demonstrated in rodents by donor-reactive T_M s generated through homeostatic proliferation. In an experimental model where recipients were devoid of endogenous T cells, adoptive transfer of naïve syngeneic splenocytes resulted in the rapid homeostatic expansion of the transferred cells and acquisition of a memory phenotype. Importantly, when these animals were challenged with allogeneic skin grafts, the transplants were rejected despite treatment with costimulation blockade [34]. These data provide direct experimental evidence that donor-reactive memory T cells can be generated by homeostatic mechanisms.

Effects of Immunosuppressants on Memory T Cells

Given that T_{EM} have unique properties, it is not surprising that they also have characteristic sensitivities to various immune therapeutics that distinguish them from naïve cells.

T cell depleting agents

Many agents are used in clinical transplantation to intentionally evoke global T cell depletion. These include polyclonal antibody preparations such as anti-thymocyte globulins (ATGs) and monoclonal antibodies specific for CD3 (muromonab) or CD52 (alemtuzumab). These agents mediate depletion through a variety of mechanisms [42,43] and while the T cell depletion that occurs following treatment with these drugs is profound, emerging evidence suggests that

T_{MS} may have some degree of resistance to depletion therapies. For example, treatment of human transplant recipients with alemtuzumab resulted in >90% T cell depletion; however, the cells that remain have been shown to contain a predominance of $CD4^+ CD45RO^+ CD62L^lo T_{EMS}$. The origin of the T_M predominance likely stems from a combination of T_M resistance to antibody mediated depletion and resultant homeostatic activation of non-depleted cells. The proliferating population is likely derived from naïve cells as these cells, being less terminally differentiated, should be expected to have greater proliferative capacity. Based on these and other studies, it is accepted that T cell depletion therapy will increase the overall frequency of T_{MS} , both due to the relative resistance of T_{MS} as well as the likely conversion of naïve to T_{MS} via homeostatic activation [44,45,46]. Recent studies have also suggested that alemtuzumab may decrease the requirement for immunosuppression by down-regulating the $CD4^+ T_{EM}$ population associated with rejection [46]. In a human cohort receiving alemtuzumab for kidney transplantation, $CD8^+$ T cells recovered to their baseline population in 6 months, while the recovery of $CD4^+$ T cells was delayed until approximately 15 months. Furthermore, the $CD8^+$ T cells that repopulated the peripheral T cell compartment by homeostatic proliferation were of immunosenescent $CD28^-/CD8^+$ phenotype, which the investigators postulated may compete for space with or even may suppress the proliferation of $CD4^+ T_{EM}$ cells [47]. Alternatively, the absence of CD28 may render these cells resistant to costimulation blockade based therapies while remaining sensitive to calcineurin inhibitors. Importantly, post-depletional T cells are T_{EM} skewed and clearly capable of mediating rejection despite exceptionally low numbers of cells without some adjuvant immunosuppressive therapy [48,49]. Thus, while their long-term characteristics may be altered in ways that influence their sensitivity to immunosuppressants, they remain immunocompetent.

Effects of T cell costimulatory pathway blockade

Costimulation is required for optimal activation of naïve antigen-specific T cells. The role of costimulation in the activation of T_{MS} is dependent on both the type of T_M and the costimulatory molecule. The CD28 pathway is one of the most important and well studied of the T cell costimulatory pathways. CD28 binds to its ligands CD80 or CD86, and propagates a positive costimulatory signal into the T cell. Agents have been developed to target this pathway. These include CTLA4-Ig, a fusion protein containing the extracellular domain of the CTLA-4 molecule, which associates tightly with CD80 and CD86 and therefore prevents CD28 ligation, and LEA29Y, a second-generation derivative of CTLA-4 Ig with increased affinity for CD86 [50]. However, evidence from several studies exists to suggest that T_{MS} are relatively independent of CD28-mediated costimulation for recall responses, thus refractory to the effects of CD28 pathway blockers. In rodents deficient in CD28, this costimulatory pathway is not necessary for the generation or recall of T_{MS} [7,51,52]. Furthermore, tolerance induction protocols based on CTLA-4 Ig have been found to be ineffective in recipients that possess cross-reactive virus-elicited donor-reactive T_{MS} . Rejection in these recipients was characterized by a lack of attenuation of donor-reactive $CD8^+$ T cell responses; however, $CD4^+$ donor-reactive T_M responses were significantly reduced following this costimulation blockade based regimen [41]. In another study, CTLA4-Ig was found to inhibit proliferation and expansion of memory $CD4^+$ T cells in response to peptide antigen challenge, with no effect on early activation [53]. Taken together, these data suggest that the type and character of the donor-reactive T_M population may influence its relative requirement for CD28-mediated costimulation upon recall. In humans and NHPs, which contain a large population of T_{MS} in their peripheral T cell repertoires, CTLA4-Ig and LEA29Y have been shown to prolong graft survival, but not to the extent evident in mouse models. Interestingly, the T_{EM} population in NHPs is largely void of CD28 and thus unlikely to be sensitive to agents targeting this pathway [54]. In humans, many $CD8^+ T_{EMS}$ are $CD28^-$ while $CD4^+ T_{EMS}$ tend to retain CD28 expression. Although systematic measurement of the effect of CD28 pathway blockade in

humans and NHPs has not been performed, it is clear that adjuvant immune modulatory agents are required to complement the effect of costimulation blockade [55,56].

The CD154/CD40 costimulation pathway is also intimately involved in the activation of T cells. In rodent models, inhibition of CD154 with anti-CD154 blocking antibodies has shown to be effective in the prevention of rejection in pre-sensitized hosts, but ineffective in sensitized hosts, indicating that CD154 may not be necessary for the activation of memory T cells [39, 40,57]. Initial CD154 blocking agents did not undergo development, and their brief investigation in human transplantation suggested a lack of efficacy [58]. As such, other modes of blocking CD154, such as blocking CD40 have been attempted, and have shown promise in long-term kidney allograft tolerance in NHPs [59]. Recent success using a fully human CD40 specific monoclonal antibody suggest that this approach has promise, but remains dependent on adjuvant therapy [60,61]. However, the direct effects of blocking CD40 and CD154 on donor-reactive T_Ms have varied depending on the model used. Using mice infected with LCMV, investigators have demonstrated that the CD4 T_M response was downregulated compared to the CD8 response when given anti-CD154 agents [62]. In contrast, using a murine cardiac allograft model, the T_M response was unchanged with anti-CD154 therapy [63]. These findings suggest that the type and rate of antigen exposure, combined with the heterogeneity of the host T_M population may demonstrate variable sensitivity to blockade of the CD154/CD40 pathway.

Another costimulatory molecule that has been shown to be important in the activation of memory T cells is the OX40 pathway, a member of the TNF receptor superfamily. This pathway has been implicated in synergistically driving the proliferation of T_Ms along with CD28-mediated costimulation [64]. In murine models, T_M mediated graft rejection was prevented when OX40 blockade was given combined with CD28/CD40 blockade, while grafts were rejected when OX40 blockade was given alone [65].

While there is a growing body of evidence to suggest that T_Ms are relatively resistant to costimulation blockade, other agents have been designed to specifically target and deplete the memory compartment based on unique enhanced expression of certain integrins, specifically CD11a and CD2 [66–68]. LFA-3-IgG1 fusion protein (Alefcept) binds to CD2 and has been shown to both prevent the activation of T_Ms and induce apoptosis, thereby decreasing the T_M population [68,69]. Alefcept is currently approved for clinical treatment of psoriasis, and its therapeutic effect is linked to its ability to deplete T_M [69,70]. Recently, alefcept has been shown to extend kidney allograft survival in NHPs when added to a CTLA4-Ig-based regimen [54]. In this study, CD4+ and CD8+ T_{EM} were shown to be specifically depleted by alefcept, and this appeared to be related to the increased expression of CD2, the target of alefcept, on T_{EM} populations. Further in vitro studies of this model examined the effect of alefcept on alloreactive cytokine producing cells and demonstrated responding alloreactive CD4+ and CD8+ T cells also exhibited increased CD2 expression, thus providing an increased available target for the effects of alefcept. This study was the first to specifically target T_Ms with the prospective intent on neutralizing cells resistant to costimulation blockade [54].

Effects of blocking signaling through the TCR and cytokine receptors

The most commonly used immunosuppressants, the calcineurin inhibitors (CNIs) (cyclosporine A and tacrolimus), target TCR-mediated signaling. These agents prevent the nuclear translocation of NFAT that is required for gene transcription of IL-2, which is important for optimal expansion and survival of T cells. The reduced requirements of T_Ms for costimulation focus a greater reliance on the TCR, and as such, CNIs have been shown to be unique among the clinically available immunosuppressants in preventing T_M proliferation and cytokine production [45]. This has been suggested to be a predominant reason why CNIs have been such important contributors to the prevention of rejection in humans. However, while

TCR signal inhibition prevents T_M activation, it also may inhibit T_M apoptosis and inhibit response contraction [71]. Thus, the CNI approach appears to have exquisite ability to prevent rejection, but may also prevent regulation and T_M contraction through apoptosis.

Inhibiting non-TCR signals in the T cell may have paradoxical influence of T_M function and in fact enhance some aspects of immunity. A recent example involves sirolimus, a clinically used immunosuppressant known to attenuate downstream signaling events through mTOR, thereby preventing G1→S transition required for T cell proliferation and population expansion. Sirolimus has been used as a replacement for CNIs with one rationale being to specifically allow TCR signaling to foster activation induced cell death. While there are few studies assessing the direct effects of these agents on the memory cell population despite their common use, it has recently been demonstrated in an infection model that sirolimus alone acts to actually increase the frequency of antigen-specific T cells that differentiate into the memory lineage [72]. In this study, mice given low-dose sirolimus following acute LCMV infection demonstrated enhanced quality and quantity of virus-specific T_M s compared with untreated controls. Similarly, NHPs given sirolimus after modified vaccinia (MVA) vaccination exhibited an increased frequency of memory T cells.

Like sirolimus, the CD25-specific monoclonal antibodies daclizumab and basiliximab also interfere with the IL-2 pathway, by binding the high-affinity α chain of the IL-2 receptor (CD25) [73]. Although CD25 is up-regulated in both naïve and T_M s upon encounter with antigen, T_M s up-regulate CD25 faster, and have been shown to subvert the blocking effects of anti-CD25 monoclonal antibodies through higher expression of the low affinity IL-2 receptor subunits (CD122 and CD132) that may support T_M proliferation and activation by ligating IL-15 in addition to IL-2 [74]. Thus, the extent to which T_M s are relevant to an individual's clinical outcome may significantly influence the relative success of CNI-sparing regimens in general, and sirolimus or anti-CD25-based regimens specifically.

Janus kinases (JAKs) are a family of cytoplasmic tyrosine kinases that regulate gene expression after signaling by cytokines. JAK3, expressed primarily in hematopoietic cells, is downstream of the common γ chain (CD132). Therefore blocking JAK3 has the potential to interrupt the signals of a broader array of cytokines used by T_M s such as IL-2, IL-7, and IL-15. Indeed, JAK3 kinase inhibitors have been shown to prevent allograft rejection in both murine and NHP models [75,76]. Further studies have proposed that prolonged graft survival observed following administration of JAK3 inhibitors could be due to blockade of signaling through the IL-7 and IL-15 receptors and thus target T_M methods for by-passing a requirement for CD25. For instance, recent investigations have suggested that IL-7 is critical both to the generation of T_M s following homeostatic proliferation, to aid the survival of T_M s, and that IL-15 is required for the generation and maintenance of anti-viral CD8 T_M s [77]. Currently one such inhibitor of the JAK3 pathway (CP-690550) is in phase II clinical trials of renal transplantation [78]. It has yet to be specifically investigated for its role in thwarting allospecific memory, but may offer a means of targeting T_M s without TCR inhibition.

Effects of blocking T cell trafficking

Given the role of T_{EM} s in initiating effector function in the periphery and the role of T_{CM} s in rapidly deriving new effectors, interruption of trafficking could have selectively potent effects of T_M function. As discussed above, inhibition of LFA-3 interactions with CD2 appear to effectively target T_M s and facilitate costimulation-based therapies. Several other agents currently under study for use in transplantation target similar processes. One such treatment is FTY720, which binds sphingosine-1-phosphate (S1P) receptor as an agonist and disables the S1P receptor from performing its function of allowing lymphocyte migration from the thymus and peripheral lymphoid tissues, effectively sequestering T cells in the lymph nodes and inhibiting them from trafficking to peripheral graft sites [26,79,80]. Blockade of CD11a also

may hold promise as an agent that targets the trafficking of T_Ms. For example, recent work has demonstrated in murine models of transplantation that anti-LFA-1 monoclonal antibodies result in attenuation of donor-reactive memory recall responses and decreased T cell trafficking to the allograft following graft placement [81,82]. The initial phase 2 testing of the anti-LFA-1 agent efalizumab in renal transplantation suggests that this agent indeed has potent inhibitory effects on protective memory in that its combination with a standard immunosuppressive regimen evoked a higher rate of EBV-associated malignancy [83]. Thus, memory inhibition is likely to require cautious pairing with other agents to avoid pathologic inhibition of protective immunity.

Conclusion

Memory T cells can pose a critical barrier to successful organ transplantation. The population of T_Ms of a given individual may vary based on the prior immune history of that patient, including the type and frequency of environmental exposures to pathogens. The alloreactivity of the T_M population may also vary based on heterologous cross-reactivity or direct prior alloantigen exposure. Regardless, the importance of a thorough understanding of the mechanisms of donor-specific T_M generation and the effects of immunosuppressive agents on these heterogeneous populations is becoming increasingly apparent. Closer attention to memory responses will likely enhance the potential to tailor immunomodulatory strategies a given transplant recipient.

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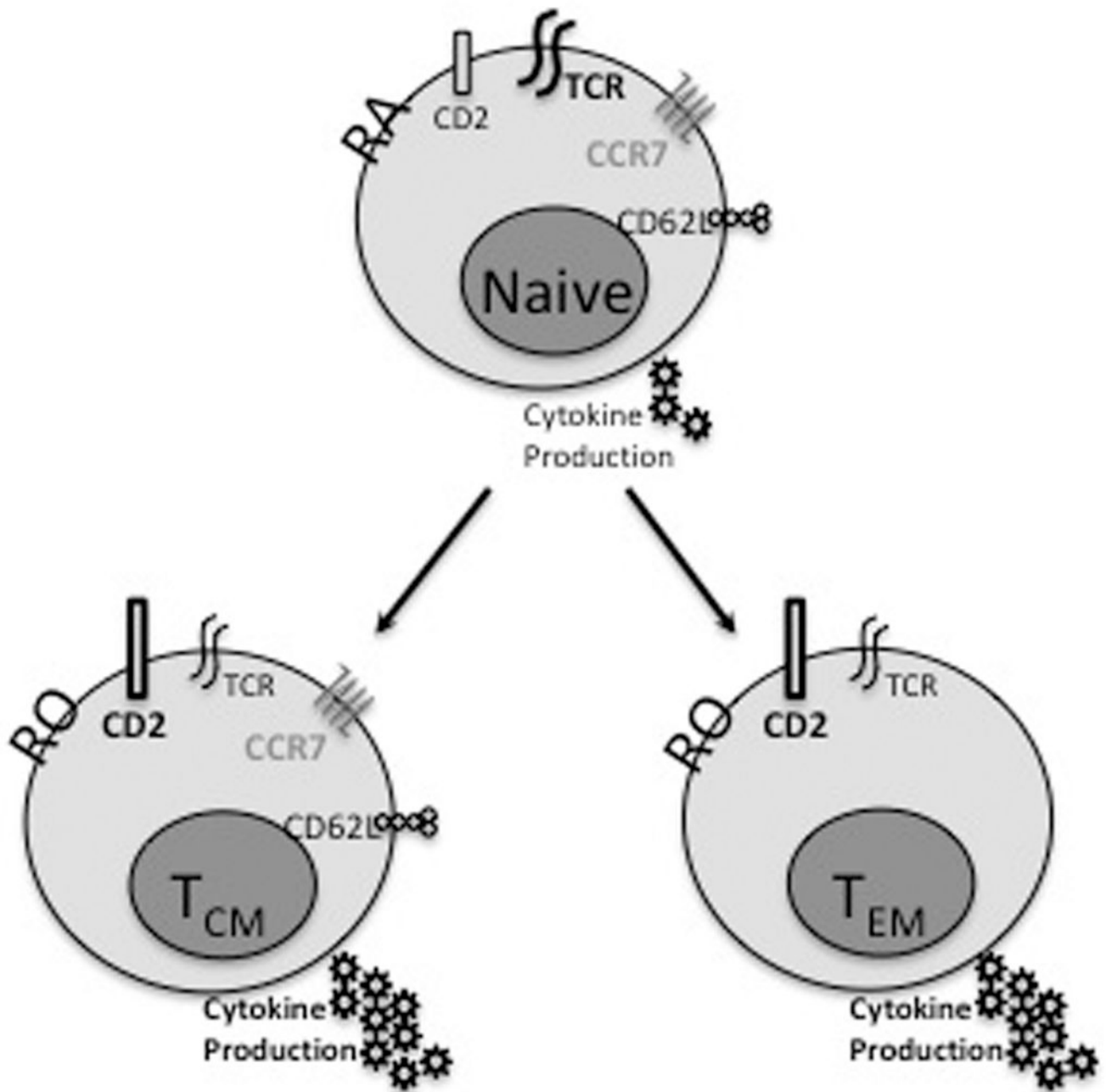


Figure 1.

Schematic of the unique characteristics of naïve T cells, central memory T cells (T_{CM}) and effector memory T cells (T_{EM}). Naïve T cells express the CD45RA isoform, have relatively high expression of the T cell receptor (TCR), low CD2 expression, and require substantial stimulation to produce cytokines and other effector molecules. With memory differentiation, T_{EM} and T_{CM} assume the CD45RO isoform, increase their expression of CD2, and reduce their expression of the TCR. Their capacity to produce cytokines is augmented. T_{CM} express CD62L and CCR7 facilitating their homing to secondary lymphoid tissues, while T_{EM} lack these molecules and reside in the periphery.