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The common γ c-cytokines and transplantation tolerance

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Summary

Transplant rejection, like tolerance, is a T cell-dependent event. There is compelling evidence to suggest that induction of transplant tolerance is an actively learned process in which T cells need to engage the alloantigens in order to learn to tolerate the allograft. A family of cytokines whose receptors use the same IL-2 receptor γ chain (also called the common γ c) plays an important role in regulating multiple aspects of the allograft response (i.e. rejection vs. tolerance). It is undeniable that γ c-cytokines can drive clonal expansion and effector maturation of alloreactive T cells, and therefore, targeting such cytokines or their receptor components remains an attractive way of blocking transplant rejection. However, we just started to appreciate that γ c-cytokines also regulate the acquisition of transplant tolerance via programming activated T cells for apoptotic cell death and via guiding the evolution of regulatory T cells. Thus, understanding precisely the role of γ c-cytokines in regulating T cell homeostasis and T cell regulation is critically important in the induction of transplant tolerance.

Key words: common γ c • cytokines • T cell regulation • tolerance • transplantation

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INTRODUCTION

A family of structurally related cytokines (i.e. IL-2, IL-4, IL-7, IL-9, IL-15, IL-21) whose receptors share the identical interleukin 2 receptor (IL-2R) γ chain is often called the γ c-dependent cytokines (Fig. 1). A key feature of γ c-cytokines is that all of them possess potent T cell mitotic activities capable of driving rapid T cell proliferation and effector differentiation^{1,39}. It is now clear that γ c-cytokines regulate several aspects of immune activation during the allograft response. They play an important role in supporting survival, proliferation, and effector function of activated T cells. Thus, blocking such cytokines often attracts considerable attention in attempting to prevent transplant rejection. However, certain γ c-dependent cytokines also play an essential role in programming activated T cells for activation induced cell death, a key process involved in T cell homeostasis and peripheral tolerance²⁵. More importantly, there is compelling evidence to suggest that some γ c-cytokines are probably indispensable in the development of regulatory T cells that may prove to be critical to long-term maintenance of a tolerant state⁴⁸. Hence, the fate of an allograft (rejection vs. tolerance) may be dependent on the balanced acts of such cytokines in affecting the life and death of activated T cells as well as in guiding the evolution of regulatory T cells. This article will review our recent understanding of γ c-cytokines in the regulation of T cell homeostasis with special emphasis on transplantation.

ROLE OF γ C-CYTOKINES IN T CELL ACTIVATION

Upon engagement of the T cell receptor along with certain costimulatory receptors, T cells often acquire exquisite sensitivity to respond to γ c-dependent cytokines. In fact, once T cells pass the stage of antigen specificity during the activation process, the fate of such activated T cells as to become armed effector cells, to commit to apoptotic cell death, or to evolve as immune regulatory T cells is regulated, to a large extent, by the γ c-cytokines¹⁹. Indeed, when activated T

cells are deprived of certain γ c-cytokines at a critical stage of cell activation, the immune activation is often aborted or the T cell activation program altered¹³.

Despite tremendous redundancy among the γ c-cytokines in stimulating T cell proliferation, not all γ c-cytokines function in the same way. In the past few years, it has been appreciated that individual γ c-cytokines exhibit certain overlaps but also distinct functional characteristics. For example, IL-2 as a T cell growth factor *in vitro* is undeniable, IL-2 also plays an indispensable role in priming activated T cells for apoptotic cell death, a feature that is not readily shared by other γ c-cytokines⁴⁴. IL-4 is instrumental in the development of T helper (Th)2 immunity¹⁸. IL-7 is absolutely required for intra-thymic T cell development⁴⁵. In immune competent hosts, IL-7 appears to be important in supporting the survival of naïve T cells in the periphery under physiologic conditions²⁷. The role of IL-9 has been rather mysterious until the generation of IL-9 deficient mice. It is now clear that IL-9 is a key growth factor for mast cells, mucosal goblet cells, and certain transformed T cells, but IL-9 has a limited role in the proliferation of primary T cells⁴². IL-15 is a recently discovered cytokine that shares many features with IL-2⁸. In contrast to IL-2, however, IL-15 acts as a survival factor for a variety of cell types and plays an essential role in supporting CD8⁺ T cells, especially the memory CD8⁺ cells^{16,50}. IL-15 is also required for development and survival of natural killer (NK) cells, NKT cells, and intra-epithelial lymphocytes. IL-21 is a new member in the γ c family and its function is just starting to be unraveled. In certain models, IL-21 can stimulate the cytolytic activity of CD8⁺ T cells and NK cells, but IL-21 by itself does not support the survival of NK cells. Interestingly, IL-21 can block IL-15 stimulated NK cell expansion¹⁴. Thus, IL-21 may be at the interface between innate immunity and acquired immunity. However, a recent report suggested that IL-21 is a Th2 cytokine and capable of inhibiting interferon γ production by Th1 cells⁴⁷. In this regard, the precise relationship between IL-21 and IL-4 remains completely unknown. Clearly,

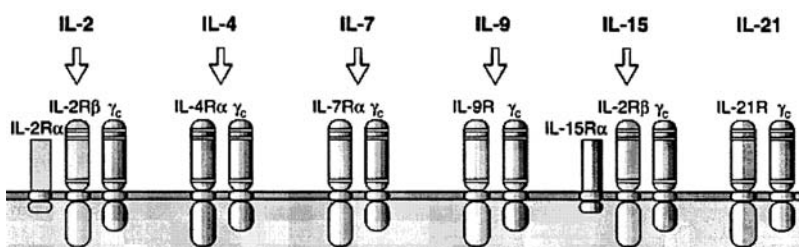


Figure 1. The common γ c as a shared receptor component. The receptors for IL-2 and IL-15 consist of a private α chain but share the IL-2R β chain and the γ c chain. The receptors for IL-4, IL-7, IL-9, and IL-21 are composed of a distinct α chain, which defines the cytokine specificity, and the common γ c chain.

a balanced act of γ c-cytokines is undoubtedly important in shaping up the nature of the T cell response.

It should be emphasized that not all γ c-dependent cytokines are produced by the same kind of cells. IL-2 is produced primarily by activated T cells, especially CD4⁺ T cells and production of IL-2 is tightly regulated at the transcriptional level. Several key costimulatory molecules including CD28 play an important role in promoting IL-2 production. IL-4 is mainly from Th2 cells, NKT cells, and some unconventional cell types such as eosinophils. Interestingly, transcriptional activation of the IL-4 gene requires cell cycle progression, suggesting that cell cycle regulators may contribute to the IL-4 gene expression². IL-21 is also a T cell product, the precise mechanism regulating IL-21 expression remains largely unknown. On the other hand, IL-7 and IL-15 are not produced by T cells. Epithelial cells, stromal cells, and to a certain degree the platelets, are the major source of IL-7. Similarly, a wide variety of cell types including macrophages, dendritic cells, endothelial cells, myocytes, keratinocytes, and even the neurons can produce large amount of IL-15⁴⁰. Furthermore, production of IL-15 is regulated not only at the transcriptional level but also at the post-transcriptional level, and a significant proportion of IL-15 is membrane bound rather than secreted⁴⁰. Thus, the *in vivo* availability and accessibility of IL-7 and IL-15 to T cells are likely to be different as compared to the T cell derived cytokines. Moreover, the non-T cell origin of IL-7 and IL-15 also suggests that the *in vivo* availability of IL-7 and IL-15, as opposed to IL-2 and IL-4, is not strictly dependent on T cell activation *per se*. Therefore, it is likely that activation of T cells can affect the nature of the cytokine milieu and the nature of the T cell response can be influenced by distinct γ c-cytokines.

It is quite interesting that the cell surface receptors for the γ c-cytokines are polymeric structures (Fig. 1). The receptors for IL-2 and IL-15 consist of a private α chain that defines the binding specificity for IL-2 or IL-15 and a shared IL-2R β chain and γ c chain. The receptors for IL-4, IL-7, IL-9, IL-21 are composed of a distinct α chain and the γ c chain³⁹. Different receptor components can be expressed individually on T cells but they have to associate with each other in order to function as a high affinity receptor complex. Thus, differential receptor configurations on the surface of activated T cells certainly affect their responsiveness to different cytokines. We have recently shown using an *in vivo* cell division model that during the initial 4 to 5 cell divisions, activated T cells express the IL-15R α chain but not the IL-2R α chain²⁰. As the shared β chain and the γ chain are constitutively expressed, T cells at the early activation

stage are primarily IL-15, but not IL-2, responsive. However, IL-2 appears to be important in the later stage of T cell response as the IL-2R α chain is highly expressed after 5 cell divisions²⁰. The precise mechanisms that control such differential configuration of cytokine receptors on actively cycling T cells are completely unknown. Nonetheless, the T cell activation status, availability of certain cytokine *in situ*, accessibility of receptor subunits, and possibly the nature of costimulatory signals may all contribute to the selective responsiveness of activated T cells to different γ c-cytokines.

THE γ C-CYTOKINES IN LIFE AND DEATH OF EFFECTOR T CELLS

The ability of T cells to be able to proliferate and rapidly expand after antigenic encounter is truly remarkable. In certain models, antigen specific T cell clones can expand over 100 times of their original size within just a matter of days following antigen exposure¹⁹. However, overwhelming majority of such expanded T cells (> 90%) have to die via a process called apoptosis when the offending antigen is cleared, as the immune system simply does not have the space to accommodate all the activated T cells from every immune response. T cell apoptosis also helps the immune system to resets its balance, a process called T cell homeostasis. Failure to control apoptosis of activated T cells is certainly devastating to the hosts. It is clear now that certain T cell growth factors (TCGFs) are fundamentally important in regulating multiple aspects of this process.

A paramount example in this regard is the striking phenotype of IL-2 knockout mice. Despite the mounting evidence of IL-2 as a key growth factors *in vitro*, mice deficient for IL-2 are not immunodeficient but rather develop a profound lymphoproliferative syndrome characterized by continued expansion of activated T cells in the periphery¹¹. Similar phenotype is present in mice deficient for the IL-2R α chain or the IL-2R β chain. Thus, IL-2 appears to perform a dual role in the T cell response, a growth factor for T cell proliferation and also a death factor for activated T cells. It remains unclear how IL-2 does this opposing functions. IL-2 by itself is not directly cytotoxic, but it can modify the survival and/or the death program of activated T cells, and cell cycle transition seems to be required in this regard. There are two general pathways by which T cell apoptosis can be initiated, i.e. engagement of cell surface death receptors (e.g. Fas) or deprivation of survival signals to activated T cells (e.g. downregulation of Bcl-2 family proteins)⁴³. IL-2 seems to affect both of these apoptotic pathways. It has been shown that IL-2 can transcriptionally shut-

down the expression of FLICE-like inhibitory protein (FLIP), which is downstream of Fas³¹, allowing Fas to recruit and activate caspases to execute the apoptotic process¹². We have recently shown that IL-2 can also bring down the surface expression of γ_c , and therefore, rendering cells susceptible to apoptosis by decreasing Bcl-2 expression²⁰. Interestingly, γ_c down-regulation occurs only after certain numbers of cell divisions *in vivo* and also requires functional IL-2²⁰.

Whether cytokines other than IL-2 (i.e. IL-4, IL-7, IL-9, IL-15, IL-21) can program activated T cells for apoptosis remains enigmatic. Under certain conditions, high levels of IL-4 and IL-7 have been shown to induce apoptotic death of activated T cells *in vitro*⁴⁹, however, their roles *in vivo* are less clear. IL-4 knockout mice do not have apparent defects in apoptosis, suggesting that IL-4 has a minimal role in priming T cells to undergo activation induced cell death *in vivo*¹⁸. Although IL-7, like other γ_c -cytokines, stimulates vigorous proliferation of activated T cells, IL-7 has been shown to protect lymphoid cells from undergoing apoptosis¹⁶. Interestingly, transgenic expression of IL-9 in mice induces formation of lymphomas³², suggesting that IL-9 has intrinsic features of promoting tumorigenesis rather than priming for apoptosis. IL-15 binds to a trimeric cell surface receptor that shares both the IL-2R β chain and the γ_c chain. IL-15, like IL-2, is remarkably potent in supporting T cell proliferation *in vitro*. In contrast to IL-2, however, IL-15 appears to prevent apoptosis and promote cell survival³. Indeed, mice deficient for IL-15 or IL-15R α chain have a lymphopenic phenotype^{15,26}, which is diametrically opposed to that of the IL-2 deficient mice. On the other hand, over-expression of IL-15 as a transgene in mice leads to the eventual development of lymphocytic leukemia⁹, further supporting the claim that IL-15 is an important survival factor. The precise function of IL-21 *in vivo* and its relationship with other γ_c -cytokines remain to be clearly defined. However, certain evidence suggests that IL-21 can support effector function of T cells and NK cells but does not support the survival of NK cells¹⁴, how such different functions are accomplished by IL-21 is entirely unknown.

Clearly, regulation of T cell survival and T cell apoptosis is a delicate teamwork and a balanced act of all γ_c -dependent cytokines is of central importance. Thus, abnormality of either one of them can have a profound impact on the homeostasis of the immune system.

ROLE OF γ_c -CYTOKINES IN REGULATORY T CELL FUNCTION

Regulatory T cells, particularly the CD4⁺CD25⁺ regulatory T cells, have received tremendous attention

recently. In the transplantation setting, generation of regulatory T cells that can suppress the alloreactive T cells escaping the initial tolerizing therapy is undoubtedly important in the maintenance of a tolerant state over time. In fact, active immune suppressive mechanisms involved in allograft tolerance have been repeatedly demonstrated in several transplant models⁵. However, the identity of suppressor cells, and more importantly, the signals and signs required for their development and function have defied our full understanding. As T cells involved in the allograft rejection are extremely diverse, it is likely that the regulatory cells that actively suppress the alloimmunity are also heterogeneous.

As mentioned above, the cell type that received most attention now is the CD4⁺CD25⁺ regulatory T cells. CD4⁺CD25⁺ cells are originated in the thymus and exported to the periphery where they function as potent suppressor cells³⁵. CD4⁺CD25⁺ cells are clearly important in control the development of autoimmune diseases and in certain models maintaining transplant tolerance^{36,41}. Although the precise mechanisms concerning their development and their acquisition of antigen specificity in mediating suppression are poorly characterized, IL-2 seems to be essential for the functional integrity of CD4⁺CD25⁺ T cells. IL-2 plays an important role in development, survival and expansion of such cell type, as CD4⁺CD25⁺ T cells are defective in IL-2 deficient and IL-2R α deficient mice¹⁰.

In certain transplant models, the tolerant status can be adoptively transferred by T cells to a new cohort of naïve animals, a phenomenon called “infectious allograft tolerance”³⁰. Adoptive transferring of the tolerant status is critically dependent on CD4⁺ T cells. Several important points should be emphasized in this model. First, suppressor T cells developed in the primary tolerant hosts are extremely effective in suppressing the alloreactive effector T cells in the secondary hosts. Second, such suppressor T cells can convert naïve T cells into suppressor T cells in the secondary hosts. Third, the suppressor mechanism once established is a robust and self-perpetuating process. In some models, suppression is probably mediated by IL-4 dependent immune deviation. For example, the ability of tolerant CD4⁺ T cells to transfer allograft tolerance to secondary hosts can be blocked by neutralizing IL-4 during the induction of tolerance in the primary recipients⁷, suggesting a critical role of IL-4 in regulating this process. Recently, IL-15 has been implicated as a key cytokine in survival and expansion of memory T cells²⁹. Whether IL-15 can also stimulate the development of T cells with regulatory properties is unknown. IL-15 is clearly

important in development and probably also in survival of NKT cells that are capable of suppressing certain autoimmune diseases^{28, 34}. However, their role in transplant tolerance remains to be determined.

ROLE OF γ C-CYTOKINES IN ALLOGRAFT REJECTION

The role of IL-2 in acute allograft rejection, a process often associated with a Th1 type immune activation, is compelling. However, IL-2 deficient mice remain capable of vigorously rejecting the MHC mismatched allografts with a kinetics that is comparable to the wild type controls. IL-4 deficient mice also readily reject islet and cardiac allografts. Moreover, mice deficient for both IL-2 and IL-4, two classical T cell derived cytokines, also vigorously reject islet allografts²⁴. Rejection in this model is clearly a T cell dependent process as rejection of the islet allografts in IL-2/IL-4 double knockout mice showed a classical mononuclear infiltrate. Rejection in this model was also associated with intra-graft expression of cytotoxic T lymphocyte (CTL) gene transcripts, and graft survival can be prolonged by targeting T cells using the anti-CD3 monoclonal antibody (mAb). IL-7 and IL-15, the non-T cell derived cytokines, are highly expressed during IL-2/IL-4 independent allograft rejection²⁴. The critical role of IL-7 and IL-15 in supporting the IL-2/IL-4 independent rejection is further highlighted by the finding that treatment of IL-2/IL-4 double knockout mice with anti- γ C mAbs can markedly prolong the allograft survival. In a minor antigen mismatched heart transplant model, blocking the IL-15/IL-15R using the soluble IL-15R α chain induced prolonged allograft survival, albeit this protocol had minimal effect in prolonging fully MHC mismatched allografts³³. Interestingly, despite expression of other γ C-cytokines in rejecting allografts, IL-9 gene transcripts were conspicuously absent. These findings suggest that regulation of IL-9 is distinct from that of IL-2, IL-4, IL-7 and IL-15, and that IL-9 is unlikely to play a key role in acute allograft rejection.

Similar to our findings in the mouse, we and others have routinely found IL-7 and IL-15, but not IL-9, gene transcripts in rejecting renal allografts from patients under conventional immunosuppression³⁷. As IL-7 and IL-15 expression is often resistant to cyclosporin A, it is likely that IL-7 and IL-15 may play an important role in chronic allograft rejection in humans under conventional immunosuppression. Clearly, the γ C-cytokines are remarkably redundant in supporting T cell activation and acute allograft rejection, and absence of either one of them is unlikely to have a significant impact on the rejection response, especially across the full MHC barriers.

ROLE OF γ C-CYTOKINES IN ALLOGRAFT TOLERANCE

In an effort to understand the role of γ C-dependent cytokines in the induction of transplant tolerance, we and others have found that it is extremely difficult, if not impossible, to tolerize IL-2 knockout mice using tolerizing protocols that are not inherently lymphoablative. In an islet transplant model, treatment of IL-2 knockout mice with rapamycin, anti-CD3, or anti- γ C mAbs failed to induce stable engraftment and all islet allograft were eventually rejected. In contrast, rapamycin treated wild type control mice experienced long-term islet allograft survival⁴⁶. Similar finding was reported in a cardiac transplant model in which costimulatory blockade uniformly created a long term cardiac allograft survival in wild type control mice but it failed to do so in IL-2 knockout mice⁶. Hence, IL-2 is not required for rejection but seems to be indispensable for tolerance induction.

A defect in apoptotic death of activated T cells seems to contribute to the failure of tolerance induction in the IL-2 deficient mice. This claim is strengthened by the finding that mice with transgenic expression of Bcl-XL, a potent anti-apoptotic molecules, in the T cell lineage are also resistant to the induction allograft tolerance using the costimulatory blockade protocol⁴⁶. In fact, T cells from the Bcl-XL transgenic mice have incredible longevity both *in vitro* and *in vivo* regardless of their activation status. As a consequence of such survival advantage, treatment of Bcl-XL transgenic mice with donor specific transfusion plus CTLA-4Ig or anti-CD40L mAb failed to prevent cardiac allograft rejection while this protocol consistently produced uniform allograft survival in the wild type control mice⁴⁶. We also established the same principle in conventional mice. Treatment with costimulatory blockade (i.e. CTLA-4Ig and anti-CD40L) plus cyclosporine, a protocol that completely prevented T cell activation and T cell apoptosis, also precluded the induction of skin allograft tolerance whereas promoting T cell apoptosis by the provision of rapamycin to the costimulatory blockade protocol facilitated tolerance induction²³. Despite the close relationship between IL-2 and the functional integrity of the Fas triggered apoptotic pathway, allograft tolerance can be readily induced in Fas deficient mice²², suggesting that an IL-2 driven apoptotic process that is independent of Fas is critically important in these models, although the precise mechanisms remain to be clearly defined.

The role of other cytokines in the induction of allograft tolerance is less well studied. Allograft tolerance can be induced in the absence of IL-4. However, using certain immunosuppressive protocols, especial-

ly those that can directly kill host lymphocytes, induction of allograft tolerance appears to be difficult in IL-4 deficient mice³⁷, suggesting that in certain but not all models IL-4 may be required for the induction transplant tolerance. As IL-4 is instrumental in the development of Th2 cells and a Th2 environment has been suggested to be permissive for tolerance induction, the requirement of IL-4 may be related to the activation of Th2-like immune regulatory network that facilitate tolerance induction. Indeed, in a cardiac transplant model, neutralizing IL-4 at the time of transplantation blocked the adoptive transfer of tolerance to the secondary hosts, albeit stable cardiac allograft survival in the primary hosts was not affected⁴. Similar finding was reported in other transplant models⁴⁸. In an islet transplant model, blocking the common γ c, the shared receptor element by all known γ c-cytokines, induced precipitous T cell apoptosis and stable allograft tolerance²¹. However, the individual role of IL-7, IL-15, and IL-21 in transplant tolerance is still unknown, and further research in this area is warranted in the future.

CONCLUSIONS

Clearly, the impact of γ c-cytokines on T cell activation, T cell apoptosis, and the evolution of regulatory T cells is far more complex than initially anticipated. As transplant rejection entails the direct recognition of foreign MHC molecules and the activation of an unusually large alloreactive clonal size, we believe that an apoptotic process initiated by some γ c-cytokines during T cell activation is critically important in the induction peripheral allograft tolerance with regimens that do not directly kill host lymphocytes. The initial apoptotic process may also promote or foster selective survival of regulatory T cells that maintain the tolerant status over time. Clearly, a balanced act of γ c-cytokines is critically important in this regard. A detailed understanding of γ c-cytokines in the allograft response will undoubtedly lead to the design of more effective strategies to induce long-term engraftment in the clinic.

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