REVIEW

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Interleukin 7 receptor α as a potential therapeutic target in transplantation

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Abstract

Drugs targeting memory lymphocytes may allow for a better control of rejection in transplantation, particularly in immunized patients. In this article the rationale of targeting interleukin 7 receptor α (IL-7Rα), a molecule expressed by both memory and naive T cells, is reviewed in the context of transplantation. Whereas naive T cells are partly responsible for acute rejection and are targeted by current immunosuppressive drugs that block costimulatory signals (cyclosporine A, anti-CD3 antibody, anti-CD52 antibody, anti-thymocyte globulin, etc.), memory T cells are resistant to costimulation blockade. As such, memory cells are an obstacle to experimental tolerance induction and may be involved in chronic rejection. There is thus much scientific interest in developing molecules able to target these cells. The role of the IL-7/IL-7Rα pathway in transplantation rejection has been suggested by the effect of an anti-IL-7 monoclonal antibody which, when associated with costimulation blockade, prolonged heart allograft survival in mice. Here the hypothesis that targeting IL-7Rα would preserve effector T cells that are less dependent on IL-7 for survival while sparing regulatory $CD4^+$ $CD25^{high}$ $IL\text{-}7R\alpha^{low}$ T cells is discussed. An anti-IL-7Rα antibody could also help achieve allograft tolerance by reducing alloreactive cells.

Key words: interleukin 7 receptor alpha, memory cells, TCR signaling, allograft tolerance.

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INTRODUCTION

Although recently developed immunosuppressive drugs have substantially decreased the incidence of acute rejection, inhibiting memory T-cell expansion in order to control chronic rejection remains a challenge in transplantation. In addition, long-lived memory cells, which enable the generation of more rapid and vigorous responses upon re-exposure to pathogens, are a barrier to allograft tolerance (Brook et al. 2006). In the 1990s, Akbar and coworkers described host memory T cells displaying reactivity against donor kidney alloantigens in patients with acute rejection (Akbar et al. 1990). This was supposed to result from degeneracy of the memory T-cell repertoire (TCR), resulting in heterologous immunity (alloreactivity of the TCR to unrelated antigens as a result of previous immunological exposure) (reviewed in Adams et al. 2003a; Adams et al. 2003b). Moreover, current "induction" drugs, which are responsible for a drastic lymphopenia in graft recipients, may induce a wave of memory-like cells (homeostatic proliferation) with exacerbated functions against donor alloantigens (Moxham et al. 2008). These lymphopenia-induced memory cells are resistant to any costimulation blockade (Yang et al. 2007) and share characteristics of memory T cells that make them hard to control. For example, they have a low activation threshold, resistance to depletion therapies using antibodies (Gallon et al. 2006; Pearl et al. 2005; Sawchuk et al. 1995) and apoptosis (Wu et al. 2004), and display more robust recall responses that require minimal or no costimulation (Cho et al. 2000). Although memory T cells are involved in protecting against re-encountered pathogens or persistent viral infections, developing strategies that target this subpopulation rather than the entire T-cell pool would help conserve at least some immune responses in graft recipients. Such strategies would avoid the development of post-transplant infection (Fishman and

Rubin 1998), malignancies, and lymphoproliferative diseases (Dantal et al. 1998) consecutive to severe lymphopenia. One potentially relevant way of targeting the memory T-cell pool would be to target the interleukin 7 receptor (IL-7R)α chain (or CD127).

In 1988, Namen et al. discovered a 25-kD murine proliferation factor for precursor B cells that they called IL-7 (Namen et al. 1988). IL-7 is produced by stromal tissues, including bone marrow, as well as thymic epithelial and intestinal epithelial cells. IL-7 is also expressed by a murine fibroblast line, a hepatoma cell line, the spleen, and the kidney (Sakata et al. 1990). Studies in mice and humans have shown that IL-7 induces proliferation and has anti-apoptotic effects in thymocytes (Conlon et al. 1989; Napolitano et al. 2003) and also promotes the survival of peripheral memory T cells in mouse models (Kondrack et al. 2003; Schluns et al. 2000).

First studied in mouse, the receptor for IL-7 is a heterodimer made up of an α subunit (IL-7Rα or CD127) and the cytokine receptor-common γ chain (γc). IL-7Rα also forms part of the heterodimeric complex of thymic stromal-derived lymphopoietin (TSLP), an IL-7-like cytokine composed of IL-7Rα and the TSLP receptor (Park et al. 2000). In the mouse, IL-7Rα is present on thymocytes, cell lines of the T-cell lineage, cell lines of myelomonocytic origin, and various tissues (bone marrow, lymph node, and spleen) (Park et al. 1990). IL-7Rα was also shown to be expressed on human vascular endothelial cells, affecting cell proliferation in a manner independent of vascular endothelial growth factor and basic fibroblast growth factor (Denis et al. 1996; Dus et al. 2003). There are no data about IL-7Rα expression on murine endothelial cells, although murine IL-7 activity towards murine endothelial cells has been reported (Denis et al. 1996). B-cell lymphopoiesis in the bone marrow of IL-7$^{-/-}$ mice was shown to be blocked at the pre-B-cell stage and these mice display reduced numbers of splenic mature B cells (von Freeden-Jeffry et al. 1995). IL-7$^{-/-}$ and IL-7Rα$^{-/-}$ mice display decreased thymic cellularity with impaired T-cell expansion and maturation as well as peripheral lymphopenia, with a more severe phenotype in IL-7Rα$^{-/-}$ mice (Moore et al. 1996; Peschon et al. 1994; von Freeden-Jeffry et al. 1995). As reported by Kondrack et al. and Carrio et al., mouse memory cells bear IL-7Rα and depend on IL-7 for their survival (Carrio et al. 2007; Kondrack et al. 2003; Moore et al. 1996). However, the involvement of the IL-7/IL-7Rα pathway in the functional differentiation of effector T cells into memory T cells is not yet clear (Klonowski et al. 2006), and at least for CD8$^+$ memory T cells the hypothesis is that IL-7Rα provides a survival advantage without being involved in the differentiation process itself (Carrio et al. 2007). Some phenotypic differences between IL-7$^{-/-}$ and IL-7Rα$^{-/-}$ mice may be explained by the involvement of IL-7Rα in the receptor complex of TSLP, which is also implicated in early thymocyte expansion (Jiang et al. 2007). In fact, mutation of IL-7Rα blocks the IL-7 and TSLP pathways, whereas in IL-7$^{-/-}$ mice the TSLP pathway is always functional. In humans as in mice, NKT cells are dependent on IL-7R signaling for their proliferation (Boesteanu et al. 1997; de Lalla et al. 2008). IL-7Rα was shown to be not required for the development of dendritic cells and monocytes in mouse as IL-7Rα knockout bone-marrow donor cells efficiently reconstituted the myeloid compartment of irradiated mice (Takeuchi and Katz 2006). However, TSLP, sharing the IL-7Rα with IL-7, was shown to enhance the maturation of human CD11c$^+$ blood dendritic cell and T-cell costimulatory capacities, involving IL-7Rα in the maturation process of dendritic cells (Reche et al. 2001). Nevertheless, Reche et al. used an *in vitro* system that cannot exclude the involvement of other cytokines in human dendritic cell maturation *in vivo*, such as IL-4 and granulocyte--macrophage colony-stimulating factor (GM-CSF) (Li et al. 2000). Indeed, monocyte precursor cultured with GM-CSF plus IL-7 or GM-CSF plus IL-4 give rise to dendritic cells in an enhanced manner with IL-7 compared with GM-CSF alone, but in a more pronounced manner with IL-4, strongly suggesting a role for other cytokines and a redundant role of IL-7 in dendritic cell development (Table 1). IL-7-derived dendritic cells are also more potent in stimulating T cells than GM-CSF--derived T cells, and an antibody blocking IL-7 or IL--7Rα would go in the sense of a decreased response against allograft by blocking T-cell costimulation by IL--7-dendritic cells.

In humans, severe combined immunodeficiency due to mutations in the IL-7Rα gene is associated with lymphopenia with normal numbers of B lymphocytes and NK cells and a decreased number of T lymphocytes that fail to proliferate in response to phytohemagglutinin. These data suggest an IL-7 independence of B-cell and NK-cell development in humans and an impaired function of T cells developing despite lymphopenia (Giliani et al. 2005; Puel et al. 1998). However, this study did not evaluate the functionality of these B cells, so it cannot be excluded that they were not fully functional. Moreover, *in vitro* studies showed that IL-7 enhanced the proliferation of pro-B cells and thus that pro-B cells are responsive to IL-7 (LeBien 2000); therefore involvement of other cytokines in B-cell maturation cannot be excluded, in particular IL-21 (Konforte et al. 2009), explaining the development of B cells in IL-7Rα$^{-/-}$ severe combined immunodeficiency patients.

IL-7Rα EXPRESSION ON T CELLS AND IL-7-RESPONSIVE CELLS

In the thymus

The study by Park et al. in 1990 showed that murine thymocytes and murine cell lines of the T-cell lineage express the IL-7R (Park et al. 1990). Kim et al. suggested IL-7 to be a trophic and survival factor rather than a growth factor in murine T-cell development, given that the cell division of double-negative thymocytes decreas-

Table 1. IL-7Rα expression in human and murine tissues and cellular subtypes

Cellular subtype, tissue	IL-7Rα expression in human	IL-7Rα expression in mouse
Pre B cells	–	+
Pro B cells	+	+
Mature B cells	–	–
TN pro T1	+	+
TN pro T2	+	+
TN pro T3	+	+
TN pro T4	+	+
DP	+	+
NK cells	–	–
NKT cells	+	+
Macrophages	+	+
Dendritic cells	–	–
Endothelial cells	+	+
Stromal cell	+	+
Bone marrow	+	+
Lymph node	+	+

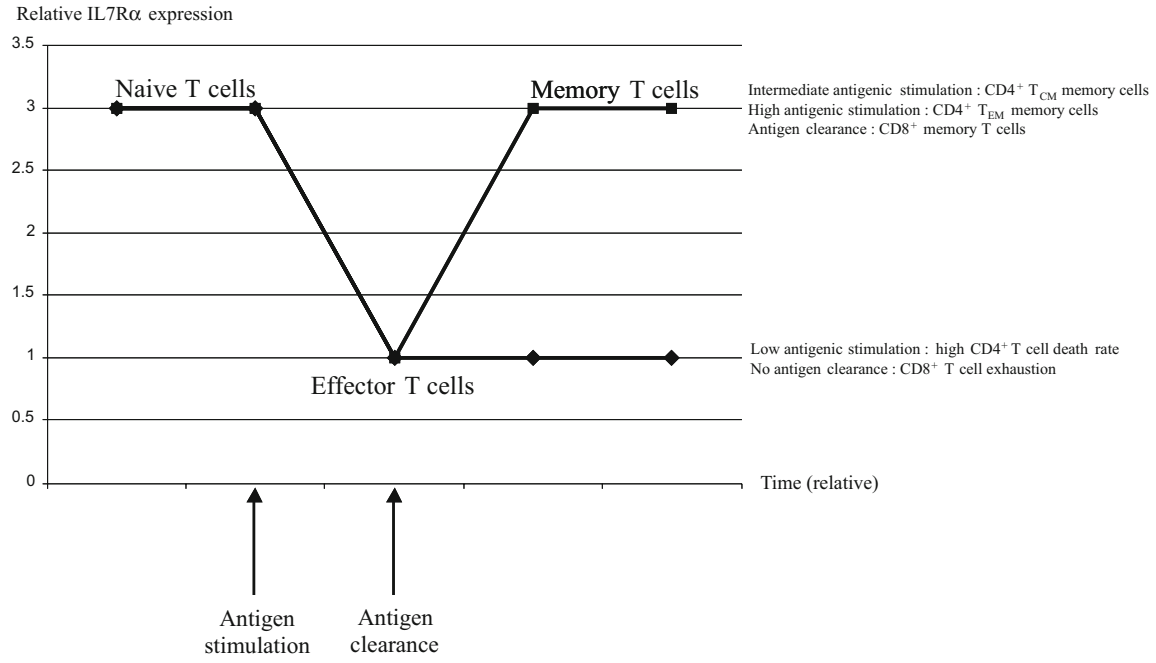
Abbreviations: TN pro T1 – triple negative ($CD3^-$ $CD4^-$ $CD8^-$ $CD44^+$ $CD25^-$ cells); TN pro T2 – triple negative ($CD3^-$ $CD4^-$ $CD8^-$ $CD44^+$ $CD25^+$ cells); TN pro T3 – triple negative ($CD3^-$ $CD4^-$ $CD8^-$ $CD44^-$ $CD25^+$ cells); TN pro T4 – triple negative ($CD3^-$ $CD4^-$ $CD8^-$ $CD44^-$ $CD25^-$ cells); DP – double positive ($CD4^+$ $CD8^+$ cells).

es upon culture with IL-7, with most cells blocked at the G1 stage (Kim et al. 1998). Studies by Peschon et al. and Moore et al. showed that in IL-7Rα$^{-/-}$ and IL-7$^{-/-}$ mice, total thymic cellularity was significantly reduced between, respectively, 0.01% to 10% and to 5% compared with wild-type (WT) mice. In these null mice, lymphopenia with thymic cellularity of less than 1% of that observed in WT mice blocks the development of thymocytes at the double-negative stage in IL-7Rα$^{-/-}$ mice and blocks the development of TCRγδ T cells in IL-7$^{-/-}$ mice. These results suggest that IL-7/IL-7Rα is involved in the transition of thymocytes from the double-negative to the double-positive stage (Moore et al. 1996; Peschon et al. 1994). Moreover, the positive selection of double-positive cells to single-positive cells leads to an up-regulation of the IL-7Rα$^{-/-}$ common γ_c chain complex and to an IL-7-dependent expansion of positively selected single-positive thymocytes (Hare et al. 2000). However, this trophic role of IL-7 is not clearly defined, given that IL-7Rα$^{-/-}$ mice display double-positive thymocytes despite a marked block in thymocyte differentiation at the double-negative stage. Moreover, IL-7Rα$^{-/-}$ double-positive cells are able to differentiate into single-positive cells, but with decreased proportions of single-positive cells compared with WT mice (Hare et al. 2000). It has also been shown that almost 50% of CD34$^{-/-}$ murine thymocytes, including double-positive thymocytes, express IL-7Rα, although the IL-7-mediated stimulation is blocked in these cells. This suggests a basal expression of IL-7Rα on different thymocyte subtypes, but with different requirements for using IL-7 (Johnson et al. 2008). Thus it may be that the maturation and expansion of thymocytes involve other cytokines, such as TSLP (Al-Shami et al. 2004) and IL-15. These cytokines are growth factors for human thymocytes (Jiang et al. 2007) and preferentially for $CD8^+$ thymocytes (Thulesen et al. 2000), likely explaining the more sizeable decrease in the number of $CD4^+$ $CD8^-$ IL-7Rα$^{-/-}$ cells than in the number of $CD4^-$ $CD8^+$ IL-7Rα$^{-/-}$ cells upon *in vitro* culture (Hare et al. 2000).

In the periphery

In humans and mice, IL-7Rα is expressed at relatively high levels on naive $CD4^+$ and $CD8^+$ T cells. IL-7Rα expression on naive human $CD4^+$ T cells increases following extended IL-7 stimulation (Swainson et al. 2006), and IL-7Rα expression on naive human $CD4^+$ and naive mouse $CD8^+$ T cells is down-regulated after TCR priming, i.e. on effector T cells (Lang et al. 2005; Lozza et al. 2008). Lozza et al. showed that low-, intermediate-, or high-strength stimulation of naive human $CD4^+$ T cells could induce IL-7Rα^{high} $CD4^+$ T cells, but that these strengths of stimulation respectively determine death, T_{CM}, or T_{EM} commitment of the IL-7Rα^{high} $CD4^+$ T cells (Lang et al. 2005; Lozza et al. 2008). In contrast, re-expression of IL-7Rα by mouse $CD8^+$ T cells is dependent on antigen clearance (Carrio et al. 2004; Lang et al. 2005). IL-7Rα^{high} $CD8^+$ T cells can potentially survive and develop more efficiently into long-lived memory T cells and can prompt greater recall responses to their specific antigen than their IL-7Rα^{low} $CD8^+$ T-cell counterparts (Fig. 1) (Kaech et al. 2003). In 1990, Grabstein et al. demonstrated that IL-7 is able to stimulate the proliferation of peripheral activated T cells in mice (resting peripheral T cells stimulated with mitogen or antigen) (Grabstein et al. 1990). Furthermore, Schluns et al. showed that IL-7 is required for the homeostatic proliferation of murine $CD4^+$ and $CD8^+$ T cells in lymphopenic hosts and for the survival of peripheral $CD8^+$ T cells (Schluns et al. 2000). Despite this IL-7 dependence of naive and memory T cells, it was shown in mice that the number of spleen and lymph node $CD4^+$ and $CD8^+$ T cells decreased only slightly (20–40%) after injection of an anti-IL-7 blocking monoclonal antibody (Sudo et al. 1993). However, contrasting results were obtained in other studies in which the rate of survival was poor in most Th2 effector cells lacking IL-7Rα in intact, lymphopenic, and class II knockout hosts (Li et al. 2003). It was also shown that the majority of $CD4^+$ memory T cells die in IL-7$^{-/-}$ hosts (Kondrack et al. 2003; Seddon et al. 2003) or in mice treated with an anti-IL-7 blocking antibody (Kondrack et al. 2003).

Human $CD4^+$ $CD25^+$ regulatory T cells express low levels of IL-7Rα (Hartigan-O'Connor et al. 2007). Recent studies in mice have shown that murine peripheral $CD4^+$ $CD25^+$ $Foxp3^+$ regulatory T cells also express low levels of IL-7Rα and that IL-7Rα$^{-/-}$ and IL-



(Adapted from Lozza et al. 2008; Lang et al. 2005)

Fig. 1. IL-7Rα expression in $CD4^+$ and $CD8^+$ T lymphocyte subsets following antigen priming.

$-7^{-/-}$ mice display reduced numbers, but normal proportions, of $CD4^+$ $CD25^+$ $Foxp3^+$ regulatory T cells despite severe lymphopenia (Bayer et al. 2008; Peffault de Latour et al. 2006).

IL-7Rα$^+$ MEMORY T CELLS AND HETEROLOGOUS IMMUNITY IN TRANSPLANTATION

Alloimmune responses remain a major problem in solid-organ transplantation, leading to both early and late graft loss. In 1990, Akbar et al. reported a high percentage of $CD45RO^+$ memory cells vs. $CD45RA^+$ naive cells in the kidneys grafts of transplant patients undergoing rejection. They demonstrated that these cells shifted from a $CD45RA^+$ to a transitional double-positive $CD45RA^+$ $CD45RO^+$ phenotype and then to a $CD45RO^+$ proliferating and activated phenotype in the grafts being rejected (Akbar et al. 1990). In these patients the role of memory cells in the rejection process was demonstrated by a correlation between the frequency of kidney graft-infiltrating memory T cells and graft rejection (Akbar et al. 1990). In other studies, the pre-transplant frequency of donor-specific memory T cells producing interferon-γ was shown to correlate with early and late acute rejection episodes (Heeger et al. 1999; Poggio et al. 2007). Moreover, Codarri et al. showed that an alloreactive IL-7Rα^{high} memory population was increased in the peripheral blood mononuclear cells of patients with chronic humoral rejection compared with stable kidney transplant patients and in stable liver transplant patients compared with healthy individuals (Codarri et al. 2007). These memory cells, which were present in equal numbers of kidney transplant patients with stable graft function and living donors before transplantation, strikingly increased as early as one month after transplantation in patients exhibiting stable graft function at one year. IL-7Rα was also predominant in the graft-infiltrating cells of patients with chronic humoral rejection (Codarri et al. 2007).

As regards acute rejection, it would be of interest to study whether the frequency of memory cell populations before transplantation or one month thereafter is also predictive of chronic allograft rejection, as memory T cells can act through heterologous immunity in both acute and chronic rejection (Adams et al. 2003a; Adams et al. 2003b). Heterologous immunity is defined by the capacity of an antigen-specific memory T cell to react to an unrelated antigen. In this concept, memory T cells that are generated in response to environmental antigens (viral infections) or alloantigens (previous blood transfusions or transplantations, pregnancies, etc.) can cross-react with allogeneic determinants. In fact, T cells specific to a given antigen can cross-react with a new antigen or with an auto- (Zhao et al. 1998) or an allo--antigen (Burrows et al. 1994) due to the degeneracy of the T-cell repertoire. For instance, lymphocytic choriomeningitis virus, pichinde virus, and murine cytomegalovirus infections in mice produce a memory cell population that protects the mouse against vaccinia virus infections (Welsh et al. 2004). Heterologous immunity has been shown to be a potent barrier to transplantation tolerance and consequently a potential cause of graft loss (Adams et al. 2003a; Bingaman and Farber 2004). Other studies have shown a strong corre-

lation between Epstein-Barr virus (EBV) and cytomegalovirus reactivation after kidney transplantation and acute rejection episodes, likely due to cross--reactivity of virus-specific memory T cells with alloantigens (Jabs et al. 2004; Reinke et al. 1994). Finally, a correlation has been described between the reappearance of virus, virus-specific memory cells, and transplant rejection. In fact, murine γ-herpesvirus-specific effector--memory cell populations generated after infection were shown to exhibit cross-reactivity with alloantigen and to interfere with tolerance induction of allogenic skin graft. Moreover, this cross-reactivity of memory T cells was shown *in vitro* in humans with EBV-specific memory T cells that could recognize and be reactivated by the given alloantigen (Burrows et al. 1994; Stapler et al. 2008). Memory cells can cross-react with alloantigens, and their frequency prior to transplantation has been shown to correlate with the appearance and severity of early and late acute rejection episodes (reviewed in Koehn et al. 2006) (Heeger et al. 1999; Najafian et al. 2002; Nickel et al. 2004).

TARGETING IL-7Rα$^+$ MEMORY CELLS AS A THERAPEUTIC TREATMENT TO PROLONG ALLOGRAFT SURVIVAL

Given the involvement of memory T cells in rejection, targeting memory T cells via IL-7Rα or IL-7 in graft recipients may be considered. This would provide a complement and/or alternative to immunosuppressive protocols that induce lymphopenia leading to memory--like T cell-induced accelerated graft rejection. The latter phenomenon was shown in a study in which homeostatic proliferation subsequent to nonspecific depletion treatments was shown to be a potent barrier to tolerance induction because it induced the differentiation of naive T cells into long-lived memory-like cells and thus increased memory functions (Hickman and Turka 2005; Moxham et al. 2008). In addition, Moxham et al. used RAG$^{-/-}$ mice as recipients and the genetically induced lymphopenia was exacerbated compared with mice in which an anti-IL-7 antibody was used. Moreover, protocols using costimulation blockade alone are efficient in delaying acute rejection, but have no effect on memory T cells (Zhai et al. 2002), as the latter are also resistant to regulation by regulatory T cells (Yang et al. 2007). An anti-IL-7Rα antibody would achieve a more efficient depletion of long-lived memory T cells, conserving at least the effector immune responses of the host against viral infections, pathogens, and the like, but inhibiting responses to graft alloantigens. In fact, effector cells are less concerned by survival via IL-7 because priming of naive T cells promotes the down-regulation of IL-7Rα and because homeostatic proliferation partly depends on IL-7 (Schluns et al. 2000).

Zhai et al. showed that sensitization by an allogeneic skin graft and treatment with CD154 monoclonal antibody was ineffective in blocking rejection of a subsequent cardiac allograft by primed/memory alloreactive CD8$^+$ T cells in mice. However, the addition of an anti-CD8 monoclonal antibody to deplete memory CD8$^+$ T cells efficiently prolonged allograft survival (Zhai et al. 2002). In a model of kidney transplantation in cynomolgus monkeys, the animals underwent a conventional immunosuppressive regimen (tacrolimus, mycophenolate mofetil, prednisone) and a conditioning regimen (total body irradiation, equine ATG, anti--CD154 monoclonal antibody) after transplantation to prepare for donor bone-marrow transplantation. Then a humanized anti-CD8 monoclonal antibody was administered for one month after donor bone-marrow transplantation. The depletion of the CD8$^+$ T-cell pool, including memory T cells, enabled mixed chimerism to be established and delayed allograft rejection (Koyama et al. 2007). One study also used this concept in a mouse model to determine if FTY720, known to sequestrate naive and newly activated T cells, also sequestrated memory CD4$^+$ T lymphocytes, preventing their encounter with alloantigens in the periphery and preventing graft infiltration (Zhang et al. 2006). This treatment, associated with a donor-specific transfusion and an anti-CD154 antibody to block the costimulation of naive T cells, also led to prolonged allograft survival (Zhang et al. 2006). However, the authors also noted a late antibody-mediated rejection, which was explained by the fact that, unlike central memory CD4$^+$ T cells, effector memory CD4$^+$ T cells express low levels of homing receptors, thereby minimizing the effect of FTY720 sequestration and allowing T-cell help for antibody-mediated rejection (Zhang et al. 2006). Another study showed that anti-IL-7 antibody together with costimulation blockade suppressed the generation of allospecific functional CD8$^+$ memory T cells, inhibited naive CD8$^+$ T-cell proliferation, and prolonged allograft survival (Wang et al. 2006). In short, these studies showed a striking implication of CD4$^+$ and CD8$^+$ memory T cells in graft loss and demonstrated that targeting CD4$^+$ and CD8$^+$ memory T cells may be an efficient way to prolong allograft survival, notably via the targeting of IL-7/IL-7Rα (Wang et al. 2006).

An advantage of targeting IL-7 or IL-7Rα would be to spare regulatory T cells, which are mostly CD4$^+$ CD25^{high} IL-7Rα^{low} T cells (Hartigan-O'Connor et al. 2007). Wang et al. used a neutralizing anti-IL-7 monoclonal antibody in a mouse cardiac transplant model and concluded that regulatory T cells were not involved in delayed allograft rejection, as they found the same frequency of regulatory T cells in the treated mice as in naive mice (Wang et al. 2006). However, it cannot be excluded that these cells play an important role in regulating the residual cells persisting after anti-IL-7 monoclonal antibody treatment (Shen et al. 2005). In contrast, Moxham et al. showed an accelerated rejection of kidney allografts in their lymphopenic mouse RAG$^{-/-}$ model due to more responsive memory T cells created through homeostatic proliferation and despite the high frequency of regulatory T cells (Moxham et al. 2008).

Nevertheless, this study did not demonstrate whether or not regulatory T cells are functional and can potentially suppress the activity of memory-like T cells created in the $RAG^{-/-}$ host. In fact, lymphopenia may have led to the creation of a high-avidity TCR memory cell population that is uncontrolled by regulatory T cells, contrary to a low-avidity TCR memory cell population (Shen et al. 2005). Moreover, it is unknown if the regulatory T cells created are specific for effector cells directed against graft alloantigen (Yang et al. 2007). It is also important to take into account the fact that naive $CD8^+$ T cells primed without costimulatory signals down-regulate IL-7Rα and re-express it after antigen clearance, even though they have become long-lived tolerized T cells (Hammerbeck and Mescher 2008). This could be of importance in protocols targeting IL-7Rα, as such potentially regulatory cells would also be deleted (Hammerbeck and Mescher 2008). However, a regulation of IL-7Rα expression by Foxp3 and an inverse correlation between Foxp3 and IL-7Rα expression were shown on different subsets of $CD4^+$ regulatory T cells, which may potentially be applicable to $CD8^+$ regulatory T cells (Liu et al. 2003; Liu et al. 2006). This suggests that anergized $CD8^+$ T cells in the study of Hammerbeck et al. are not functional regulatory T cells in that they express IL-7Rα.

PERSPECTIVES

It is possible to consider different types of therapeutic treatments involving an IL-7/IL-7Rα signaling pathway, i.e. targeting IL-7 or targeting IL-7Rα, which would block IL-7 availability by these cells in each case. However, the approach is not the same, as targeting IL-7 allows the use of IL-7Rα for other cytokines, such as TSLP (Jiang et al. 2007), and in each case it allows the survival of cells with other cytokine receptors, such as IL-15R (Berard et al. 2003). Moreover, these approaches are different as the lymphopenia induced by IL-7 targeting is less severe than that induced by IL-7Rα targeting. This would thus conserve a part of the immune response against viral infections and possibly against alloantigens. Thus further work needs to be done to identify the advantages and drawbacks of each strategy.

Another question that arises from a potential IL-7/IL-7Rα pathway blockade is the use of a blocking or a neutralizing/cytotoxic antibody against IL-7 or IL-7Rα. Indeed, a blocking antibody against IL-7 or IL-7Rα, or even a neutralizing anti-IL-7 antibody (as in the study of Wang et al. 2006), would only permit an exhaustion of the pool of $ILR\alpha^+$ cells, but would not avoid the stimulation of residual cells and thus a potential response against the allograft. A cytotoxic anti-IL-7Rα antibody would permit the whole depletion of the pool of $IL\text{-}R\alpha^+$ cells, although generating a lymphopenia that may not have more benefits than classical immunosuppressive drugs, except for the avoidance of cytotoxicity and regulatory T-cell conservation.

Finally, blocking the IL-7/IL-7Rα pathway would potentially diminish the generation of alloreactive memory cells, as obtained in the work of Wang et al. (Wang et al. 2006), but in a manner dependent on a costimulation blockade. This is an interesting finding, as IL-7Rα is not only expressed on memory cells, but also on naive cells. Thus in the study of Wang et al., lack of IL-7 should lead not only to a decrease in memory T cell population, but also to a decrease of the naive T-cell population, given the dependence of these cells to IL-7. However, IL-7 blockade alone is inefficient in prolonging allograft survival. These data suggests either that even in the absence of IL-7, naive T cells can be costimulated to develop an effector response or that naive T cells may be less dependent on IL-7 than memory T cells. It would be interesting to study in which manner these two cell subtypes are different in their dependence on IL-7 and if an $IL\text{-}7R\alpha^+$ cell blockade/depletion would gave similar results on cell behavior and allograft survival than an IL-7 blockade. Further *in vivo* studies are needed to follow $IL\text{-}7R\alpha^+$ cell contraction and confirm memory T-cell depletion after treatment with a neutralizing IL-7 or a cytotoxic IL-7Rα antibody. Most studies deal with mouse knockouts of IL-7 or IL-7Rα, which are extreme phenotypes that do not necessarily reflect the behavior of cells treated with anti-IL-7/IL-7Rα antibodies, as there can be persistent low levels of IL-7 or $IL\text{-}7R\alpha^+$ cells despite the treatment. Thus it is also important to study the profile of persistent cells in terms of activation, proliferation, and cytokine production and function. Notably, the use of a cytotoxic anti-IL-7Rα antibody may be an interesting way to bypass naive T-cell costimulation during the alloimmune response. Indeed, it would not only deplete memory T cells but also the pool of naive T cells, preventing their priming by alloantigens, their differentiation in effector T cells and finally their development in memory T cells. Such a new reagent may be a useful therapeutic agent in situations in which memory cells must be kept under control.

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