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Harnessing autophagy for adoptive T-cell therapy

"...through use of ex vivo methods of T-cell expansion that incorporate rapamycin and polarizing cytokines, autophagy can be harnessed for the generation of Th1- or Th2-type T cells that mediate increased in vivo effects upon adoptive transfer."

KEYWORDS: autophagy ■ immunology ■ rapamycin ■ Th1 ■ Th2 ■ transplantation ■ tumor

Allogeneic hematopoietic cell transplantation (HCT), which is a treatment option for leukemia and lymphoma (reviewed in [1]), represents a form of adoptive T-cell therapy (the graft-versus-tumor effect). The majority of allogeneic transplants are performed using unmanipulated donor T cells, although as we will describe below, *ex vivo*-manipulated donor T-cell therapy represents a promising new area of clinical research. In addition, in the autologous setting, *ex vivo*-expanded T cells, in particular T cells that are gene-modified to enhance tumor targeting or avoid viral infection, have been evaluated in a diversity of diseases including melanoma and sarcoma [2], leukemia [3] and HIV [4,5]. Successful adoptive T-cell therapy in part requires that the transferred T cells: differentiate into a favorable T-cell subset, which may minimally include Th1, Th2, Treg or Th17 populations depending on the clinical context [6]; and persist *in vivo* [7]. In this article, we will focus on the *ex vivo* use of rapamycin to generate functionally defined T-cell subsets that possess a favorable pattern of *in vivo* persistence after adoptive transfer. Current results indicate that rapamycin induces autophagy during *ex vivo* T-cell differentiation, with resultant T-cell acquisition of an antiapoptotic phenotype that confers *in vivo* persistence.

Autophagy is triggered during cellular stresses such as starvation, growth factor withdrawal or through rapamycin pharmacologic blockade of mTOR [8], which is a central gatekeeper of cell signaling pathways and cellular energetics. Three types of autophagy exist:

- Macroautophagy, which involves the enclosure of cytoplasm, cytoplasmic protein aggregates or whole organelles in a double membrane-bound structure that is degraded by lysosomes;
 - Microautophagy, which is the capture of cytoplasmic contents in small vesicles that bud directly into lysosomes;
 - Chaperone-mediated autophagy, which involves translocation of proteins containing a target sequence into the lysosomal membrane.
- Rapamycin primarily results in macroautophagy (hereafter referred to as autophagy) that occurs in three stages:
- Initiation, which involves the formation of an isolation membrane around the cytoplasm;
 - Maturation, where membrane expansion results in the formation of an autophagosomal double membrane;
 - Degradation in the lysosomes.

These three events are successfully orchestrated by various molecules such as the ATG series of proteins and beclin-1, with mTOR serving as a distal autophagy gatekeeper (mTOR on: autophagy inhibition; mTOR off: autophagy promotion) [9].

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Given the importance of autophagy in determining T-cell survival (reviewed in [10]), we evaluated whether rapamycin exposure during *ex vivo* T-cell culture induced autophagy and modulated T-cell survival. For these studies, we evaluated human CD4⁺ T cells that were polarized to a Th1 cytokine phenotype. Indeed, as shown in the electron microscopy images, human T cells manufactured *ex vivo* in rapamycin were replete with autophagosomes; consistent with an

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autophagy mechanism, T cells knocked-down for *Beclin-1* had reduced autophagosome formation during mTOR blockade (FIGURE 1). Initially, rapamycin was characterized as an agent that induces T-cell anergy even in the presence of costimulation [11]; in this light, it was somewhat paradoxical that the postautophagy, rapamycin-resistant T cells that we evaluated had increased *in vivo* function upon adoptive transfer into a human-into-mouse model of xenogeneic graft-versus-host disease (GVHD) [12]. In these experiments, we also found that rapamycin-resistant T cells underwent mitochondrial autophagy (mitophagy), thereby resulting in reduced T-cell mitochondrial mass but increased mitochondrial function (more stable mitochondrial membrane potential). These results extended previous findings in human cell lines, where it was observed that autophagy altered mitochondrial biology in T cells, including a reduction in mitochondrial mass [13].

“...rapamycin-resistant T cells underwent mitochondrial autophagy (mitophagy), thereby resulting in reduced T-cell mitochondrial mass but increased mitochondrial function...”

In these experiments, we also observed that *ex vivo* manufacturing in rapamycin decreased T-cell free oxygen radical formation, was associated with reduced T-cell expression of proapoptotic *bcl-2* family gene members (*BAX* and *BAK*), and yielded an antiapoptotic phenotype in rapamycin-resistant T cells [12]. We found that the antiapoptotic phenotype of rapamycin-resistant

T cells was relatively optimized: that is, such T cells had similar engraftment and persistence characteristics as transgenic T cells overexpressing antiapoptotic *bcl-2*. Importantly, we observed a nearly all-or-nothing phenomenon with respect to human T-cell engraftment and persistence in the xenogeneic GVHD model: that is, rapamycin-resistant T cells (but not control, costimulated T cells) engrafted and persisted for months after adoptive transfer. In addition, with such long-term engrafting, rapamycin-resistant human T cells vigorously engrafted upon transfer to secondary immune-deficient murine hosts [AMARNATH S, UNPUBLISHED OBSERVATION]; this latter observation is consistent with a conclusion that *ex vivo* rapamycin promoted long-term memory T-cell characteristics. These results run parallel to findings in models of malignant glioma [14] and tuberous sclerosis complex [15], where autophagy promotes tumor cell clonogenicity.

We have found that postautophagy, rapamycin-resistant T cells can be manufactured in both Th1- and Th2-polarizing conditions [16], thereby offering diverse opportunities for clinical translation. In this study, we found that *ex vivo* rapamycin promoted a T central memory phenotype; this result is consistent with the known linkage of mTOR and T-cell homing through modulation of KLF2 transcription factor levels [17] and with findings of the superior *in vivo* persistence of T central memory cells [18]. An ability to polarize towards both Th1 and Th2 phenotypes in the presence of high-dose rapamycin contrasts somewhat with results obtained using T cells genetically deficient in *TORC1/TORC2*, where mTOR status was shown to

Figure 1. Rapamycin induces autophagy in human Th1 cells. CD4⁺ T cells were costimulated with anti-CD3, anti-CD28 and cultured for 6 days in media containing rhIL2 (20 IU/ml), IFN- α 2b (1 million IU/ml) and rapamycin. (A) Resultant CD4⁺ Th1 cells expressed autophagosomes by electron microscopy. (B) Culture initiation with CD4⁺ T cells knocked-down for *Beclin-1* expression by siRNA methodology had greatly reduced autophagy.

influence T-cell polarization [19]; and, our findings differ somewhat from studies using T-cell culture without polarizing cytokine addition, where rapamycin was shown to promote Treg differentiation [20]. Because of the association of Th1-type cells with GVHD [21], rapamycin-resistant T cells may have application primarily in the autologous transplantation setting; along these lines, we have sponsored an Investigational New Drug Application with the US FDA for evaluation of rapamycin-resistant Th1 cells for immune reconstitution after autologous HCT therapy of multiple myeloma [101].

“...rapamycin-resistant T cells (but not control, costimulated T cells) engrafted and persisted for months after adoptive transfer.”

On the other hand, rapamycin-resistant Th2-type cells may have clinical translational application in the setting of allogeneic transplantation. We found that allogeneic murine Th2 cells manufactured *ex vivo* in rapamycin prevented GVHD in an IL-4 dependent manner, and when combined with T cells that induced a Th1-type response, could yield a favorable balance between GVHD protection and mediation of graft-versus-tumor effects [22]. Furthermore, we found that antiapoptotic, rapamycin-resistant Th2-type cells prevented murine hematopoietic cell graft rejection [23]; in other studies, we demonstrated that host-based therapy with rapamycin-generated Th2 cells prevented graft rejection in a rat model of cardiac transplantation [24]. Based on these results, we have initiated a pilot clinical trial of rapamycin-resistant human Th2 cells in the setting of low-intensity allogeneic HCT for therapy of refractory hematologic malignancy [102]. Initial results, presented in abstract form [25,26], indicate that this approach can promote alloengraftment and preserve antitumor effects with a low rate of GVHD.

“...rapamycin-resistant T cells can be manufactured in both Th1- and Th2-polarizing conditions ... thereby offering diverse opportunities for clinical translation.”

In conclusion, through use of *ex vivo* methods of T-cell expansion that incorporate rapamycin and polarizing cytokines, autophagy can be harnessed for the generation of Th1- or Th2-type T cells that mediate increased *in vivo* effects upon adoptive transfer. This flexibility in generation of cross-regulatory Th1/Th2 subsets indicates that this approach may have application for both autologous and allogeneic transplantation. The beneficial effects of autophagy on T-cell function are certainly complex, and are probably mediated at least in part through alterations in mitochondrial biology, apoptotic tendency and effector maturation. Further studies will be required to more fully characterize the role of autophagy for enhancement of T-cell function and to evaluate whether alternative proautophagy agents may work in a different or synergistic manner with rapamycin.

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