

T cell regulation of natural killer cells

Yann Kerdiles, Sophie Ugolini, and Eric Vivier

In light of their role in the immune response against tumors and viruses, natural killer (NK) cells represent a promising target for immunotherapy. Before this target is reached, the various mechanisms that control NK cell activity must first be identified and understood. In the past decades, studies have identified two critical processes that prevent spontaneous NK cell-mediated autoimmune activation while maximizing the efficiency of these cells during an immune response. First is the education process, whereby NK cells adapt to their environment by sensing ligands for inhibitory and activating receptors. Second is the priming phase of NK cell activation, which arms NK cells with appropriate cytotoxic molecules during inflammation. New studies now indicate that NK cell proliferation, accumulation, and activation are also under the control of regulatory T cells that restrict availability of IL-2 released by activated CD4⁺ T cells. Together with other recent studies, these data highlight the importance of the adaptive immune system in the regulation of NK cell activity.

With more than 200 clinical trials involving NK cells over the last decade, it is clear that these cells represent a promising tool in immunotherapy with a strong emphasis on cancer (Vivier et al., 2012). Indeed, many studies in mice have highlighted the potential of NK cells to eradicate developing as well as established tumors of various origins. Their antitumor potential has also been highlighted in humans in the context of hematopoietic stem cell transplantation for acute myeloid leukemia. Despite the fact that genetic depletion models have only recently become available, and that cases of human NK cell deficiencies are rare, a large body of work has demonstrated that this innate immune cell population is also critical in the control of several viral, bacterial, and parasitic infections. Nevertheless, like many other immune cell types, NK cells can also be detrimental for the

host and can contribute to the development of immune disorders. The most compelling evidence of a “dark side” for NK cells comes from studies supporting a role for pancreas-infiltrating NK cells in the development of type-1 diabetes (Feuerer et al., 2009). To develop successful NK cell-based therapies, it is critical to clearly understand how their activity is regulated. New data adds to this understanding by showing how regulatory T (T reg) cells and effector CD4⁺ T cells team up to control NK cell activation.

Ensuring appropriate activation

NK cell activation relies on the integration of signals arising from activating and inhibitory receptors. Although key inhibitory receptors recognize class I MHC (MHC-I) molecules, activating receptors recognize a range of ligands, including endogenous molecules released in situations of cellular stress, and viral proteins. This balance between inhibitory and activating signals allows NK cells to detect and kill stressed cells while sparing healthy ones. However, most inhibitory receptors are expressed in a stochastic fashion. As a consequence, the total NK cell population includes clones that will eventually express only inhibitory receptors that don't recognize

endogenous MHC-I, or even none of them, with the consequences of being potentially autoreactive by missing-self recognition. So, like T and B cells, NK cells undergo an education process to ensure that only cells expressing inhibitory receptors specific for endogenous MHC-I, and thus self-tolerant, will undergo functional maturation. However, NK cell tuning relies not only on signals from inhibitory receptors but also on activating receptors such as NKG2D, Ly49H, or KIR2DS1, which induce NK cell hyporeactivity in the chronic presence of their ligands (Vivier et al., 2008). Thus, NK cells such as T and B cells undergo an education process that adapts the threshold of NK cell reactivity to the host.

A second layer of regulation revolves around a process of functional priming controlled by the innate immune system. Until recently, NK cells were thought to be poised and ready to kill target cells on contact. We now know, however, that resting NK cells from mice and humans are not hard wired and display relatively poor effector functions (e.g., cytotoxicity and cytokine secretion) without an appropriate inflammatory context, such as dendritic cell (DC)-mediated IL-15 trans-presentation (Lucas et al., 2007; Ganal et al., 2012). IL-15 trans-presentation results in the translation of perforin and Granzyme B mRNA pools in the NK cell (Fehniger et al., 2007). Interestingly, it has been observed that mouse cytomegalovirus (MCMV) infection can lead to a breakdown in NK cell education and tolerance to self, accompanied by a drastic increase in NK cell reactivity (Sun and Lanier, 2008).

Y. Kerdiles, S. Ugolini, and E. Vivier are at the Centre d'Immunologie de Marseille-Luminy, Institut National de la Santé et de la Recherche Médicale, U1104, F-13009 Marseille, France; Centre National de la Recherche Scientifique, UMR7280, F-13009 Marseille, France; and Aix Marseille Université, UM2, F-13009 Marseille, France. E. Vivier is also at the Service d'Immunologie, Assistance Publique-Hôpitaux de Marseille, Hôpital de la Conception, F-13005 Marseille, France.

CORRESPONDENCE

Y.K.: kerdiles@ciml.univ-mrs.fr

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NK cell–T reg cell cross-talk

Recent studies have revealed a third layer of regulation that relies on the adaptive immune system, i.e., T reg cells and effector T cells. Foxp3-expressing T reg cells are critical to the maintenance of adaptive immune tolerance. Mice (e.g., *Scurfy*) and humans (i.e., patients with immunodysregulation polyendocrinopathy autoimmune enteropathy X-linked) lacking T reg cells develop rapid, widespread, aggressive, and ultimately lethal autoimmune disease (Josefowicz et al., 2012). In allogeneic transplantation settings, T reg cells suppress graft-versus-host disease (Maury et al., 2010). On the other hand, increased numbers of circulating and tumor-infiltrating T reg cells have also been observed in many cancer types and correlate with poor prognosis, and depletion of T reg cells in mice improves tumor clearance (Shimizu et al., 1999). Recent studies now show that the activity of T reg cells regulates that of NK cells. For example, the induction of NK cell effector function in response to the c-Kit tyrosine kinase inhibitor Gleevec (STI571) in patients with gastrointestinal stromal tumors, or in response to exosomes in patients with melanoma, correlated with decreased T reg cell numbers, increased anti-tumoral responses, and delayed cancer progression (Ghiringhelli et al., 2005, 2006). In patients undergoing HLA-identical blood stem cell transplantation, increased donor T reg cell numbers were associated with reduced NK cell cytotoxic activity (Trzonkowski et al., 2004b). Acute depletion of T reg cells in mice also lead to a significant increase in NK cell numbers, in line with the phenotype of Foxp3-deficient *Scurfy* mice, which harbor abnormally activated and proliferating NK cells (Ghiringhelli et al., 2005; Kim et al., 2007). In addition, whereas T reg cell transfer prevents the development of antitumoral NK cell-like activity, T reg cell (CD4⁺CD25⁺) depletion enhances NK cell-dependent tumor suppression in transplantable tumor models in mice (Shimizu et al., 1999; Smyth et al., 2006). Depletion of host T reg cells before allogeneic or haploidentical bone marrow transplantation in mice also enhances NK cell-dependent

transplant rejection, whereas co-infusing donor T reg cells prevented it (Barao et al., 2006). Finally, acute depletion of T reg cells in the BDC2.5/NOD mouse model of type 1 diabetes quickly lead to NK cell activation, IFN- γ secretion, and diabetes progression (Feuerer et al., 2009).

Collectively, these studies demonstrate that T reg cells inhibit NK cell activity in vivo. In line with these findings, in vitro experiments revealed that co-culture of NK cells with T reg cells inhibited NK cell cytotoxicity against prototypic target cells in both mice and humans (Wolf et al., 2003; Smyth et al., 2006; Trzonkowski et al., 2004a; Ghiringhelli et al., 2005), further suggesting that T reg cells can directly act on NK cells. Initial in vitro studies revealed that this T reg cell-mediated inhibition was cell contact dependent and was not affected by formaldehyde fixation of the T reg cells (Ghiringhelli et al., 2005; Smyth et al., 2006). This contact-dependent inhibition was mediated in part by membrane-bound TGF β , which is expressed on the surface of T reg cells, as blocking TGF β abolished T reg cell-dependent NK cell inhibition (Ghiringhelli et al., 2005; Smyth et al., 2006; Barao et al., 2006; Sungur et al., 2013). In addition, T reg cells derived from TGF β ^{-/-} mice were unable to inhibit NK cell functions (Ghiringhelli et al., 2005; 2006). Depletion or functional impairment of T reg cells in the mouse also leads to an accumulation of DCs expressing IL-15R α , which is associated with enhanced NK cell proliferation (Terme et al., 2008). T reg cells could therefore also indirectly control NK cells via DCs.

Effector T cells get in the mix

Three new studies now reveal a novel player in the interaction between T reg cells and NK cells. In this issue of the *JEM*, [Sitrin et al.](#) depleted T reg cells in the BDC2.5/NOD model of type 1 diabetes and found that NK cells up-regulated genes involved in proliferation, cytokine secretion, and cytotoxicity. Loss of T reg cells also caused pancreatic NK cells to down-regulate expression of genes involved in TGF β signaling. However, treatment of T reg cell-sufficient

mice with anti-TGF β antibodies did not recapitulate this phenotype, arguing against a predominant role for TGF β . Instead, pancreatic NK cell activation was associated with increased expression of genes associated with IL-2/Stat5 signaling. Additionally, the NK cell accumulation and IFN- γ secretion that was induced upon T reg depletion could be prevented by treating the mice with IL-2 antagonists. Treatment with IL-2 agonists in the presence of T reg cells was sufficient to trigger the accumulation of NK cells, IFN- γ secretion, and diabetes. In parallel, in this issue [Gasteiger et al.](#) and [Gasteiger et al.](#) found that T reg cell depletion in Foxp3.DTR mice had no effect on NK cell self-tolerance, activation by cytokines, or expression of activating receptors. However, missing-self-induced cytotoxicity was consistently enhanced in a CD4⁺ T cell- and IL-2-dependent way. As IL-2 secretion by CD4⁺ T cells is greatly enhanced upon T reg cell depletion in Foxp3.DTR mice, and IL-2 is largely confined to CD4⁺ T cells in the pancreas of BDC2.5/NOD mice, it is likely that T reg cell-dependent control of NK cell activity in vivo may require regulation of IL-2 production by activated T cells. Interestingly, IL-2 deprivation is thought to be a prominent mechanism used by T reg cells to suppress spontaneous T cell activation (Pandiyani et al., 2007; Josefowicz et al., 2012). By limiting IL-2 availability and thus controlling T cell activation, T reg cells can dynamically tune NK cell activity (Fig. 1).

IL-2 has long been used in vitro to stimulate NK cell proliferation and activation, leading to the generation of lymphokine-activated killer cells. Early IL-2 secretion by activated CD4⁺ T cells is also required for NK cells to secrete IFN- γ upon primary infection with *Leishmania* major in mice (Bihl et al., 2010). In addition, the rapid secretion of IL-2 by restimulated human memory T cells triggers NK cell activation, mimicking an antigen-specific memory NK cell response (Horowitz et al., 2010). These results reinforce the importance of IL-2 and the adaptive immune system in the regulation of NK cell activity

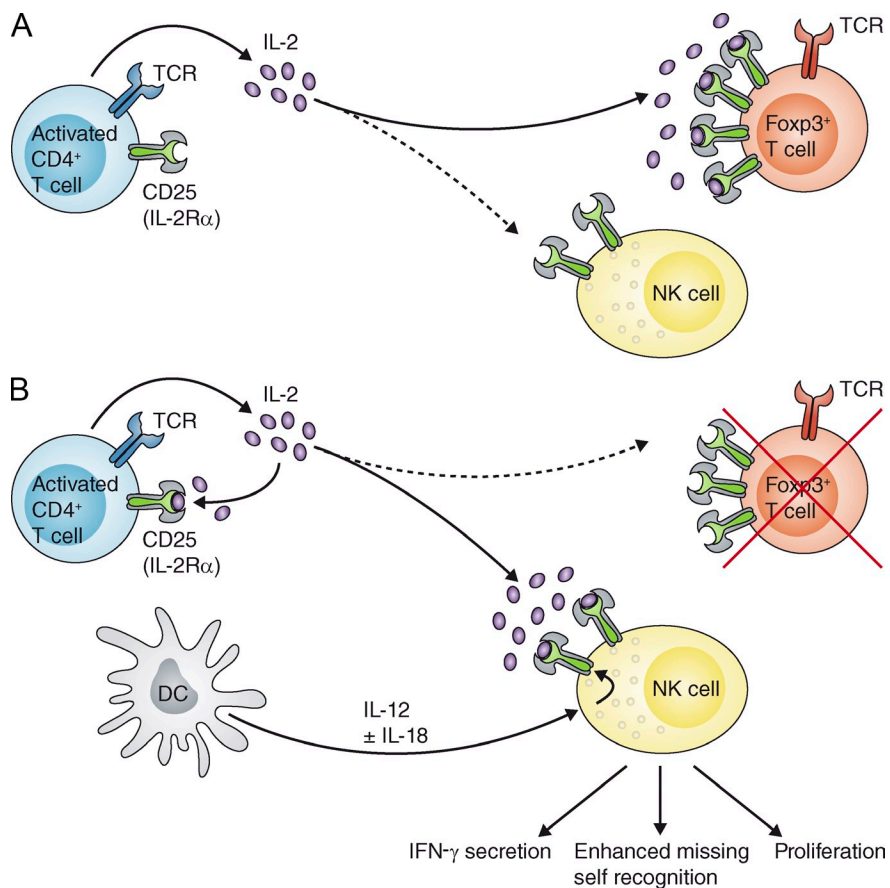


Figure 1. IL-2-dependent T reg cell-NK cell cross talk. (A) CD4⁺ T cells boost NK cell activation via IL-2, and T reg cells deprive NK cells of IL-2. (B) Upon loss of T reg cells, IL-2 becomes available, and NK cells are unleashed and may participate in tissue damage in autoimmune conditions.

in vivo, both in homeostatic and infectious conditions.

It remained unclear, however, which population of NK cells was targeted by IL-2. Indeed, resting NK cells express very low to no CD25, the specific α chain for the IL-2 receptor, and require high IL-2 concentrations to acquire cytotoxic function. Sitrin et al. (2013) found that CD25⁺ NK cells, which comprised a fairly small fraction of total pancreatic NK cells, were more potent IFN- γ producers than their CD25⁻ counterparts upon T reg cell depletion in BDC2.5/NOD mice. This suggests that the in vivo expression of CD25 is mandatory for IL-2-dependent NK cell activation. It has been shown that CD25 expression is strongly, albeit transiently, up-regulated by mouse NK cells in an IL-12 and STAT4 dependent manner during the first few days of MCMV

infection (Lee et al., 2012). In other studies, Gasteiger et al. (2013a,b) observed that CD25 is selectively expressed and up-regulated on a subset of CD27⁺ immature NK cells upon T reg cell depletion and in tumor-bearing or chronically infected mice, allowing for the IL-2-dependent accumulation of these cells. Interestingly, they also found that low-dose IL-12 enhances CD25 expression on CD127⁺ cells in vitro, whereas a combination of IL-12 and IL-18 up-regulates CD25 on all NK cells (Lee et al., 2012). IL-18 was previously shown to be required for IFN- γ secretion induced by IL-12 either alone or in combination with IL-2 (Chaix et al., 2008). In light of these data, it appears that immature CD127⁺ NK cells could bypass this IL-18 requirement. As such, CD25 up-regulation and IL-2 responsiveness of NK cells seems to be finely regulated

depending on the inflammatory context, accounting for the adaptation of the NK cell response to various immune insults and further preventing NK cell proliferation, accumulation and activation in the absence of an appropriate concomitant immune response.

The knowledge of this regulatory mechanism might be pharmacologically targeted to stimulate or dampen NK cell activity depending on the pathological context.

Our laboratory is supported by the European Research Council (THINK Advanced Grant) and by institutional grants from Institut National de la Santé et de la Recherche Médicale, Centre National de la Recherche Scientifique and Aix Marseille to CIML.

E. Vivier is cofounder and shareholder of Innate Pharma. The other authors declare no conflict of interest.

Submitted: 9 May 2013

Accepted: 13 May 2013

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