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LETTER TO THE EDITOR

Invariant natural killer T cells treated with rapamycin or transforming growth factor-β acquire a regulatory function and suppress T effector lymphocytes

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Invariant natural killer T lymphocytes (iNKT) are hybrids between innate and adaptive immune cells and exert their effector functions by binding to glycolipids. Endogenous ligands can also stimulate iNKTs to perform their functions.[1] However, they may also play a protective role, which was demonstrated in studies that exhibited their capability to elicit a regulatory potential. This novel potential was recently investigated by our group in human peripheral iNKTs.

iNKTs are characterized by the expression of a unique αβ T-cell receptor (TCR) composed of an invariant Vα24-Jα18α chain paired to a Vβ11 chain, which together are capable of recognizing glycolipids presented via the CD1d molecule.[2,3] The observation that the glycolipid α-galactosylceramide (α-GalCer) is a potent stimulator of NKT cells has provided an important breakthrough in investigating their biology. α-GalCer is presented by CD1d to the Vα24-Jα18/Vβ11 TCR which induces iNKT expansion and activation to rapidly generate large amounts of various Th1, Th2 and Th17 cytokines and chemokines.[1] Additionally, there is evidence that iNKTs expressing the transcription factor FOXP3 are equipped with regulatory functions and modulate the immune response by producing 'suppressive' cytokines.[4,5]

iNKTs with regulatory activities have been recently described in mice and have been shown to mediate immune suppression upon interaction with myeloid derived suppressor cells[6] or the production of IL-10. However, few studies have described similar regulatory iNKT cells in humans.[5] Therefore, we generated iNKT cell lines from the peripheral blood of healthy donors (10 subjects) by stimulating the cells with α-GalCer, IL-2 and IL-15 for 2 weeks. This procedure yielded an enriched (60%) iNKT population that was detected by staining with anti-human TCR Vα24Jα18-PE conjugate (BD Biosciences, San Jose, CA, USA). The iNKT cell lines were then sorted to a more than 98% pure population. The iNKT cell lines were subsequently treated with various stimuli commonly used to generate *in vitro* regulatory T cells (Tregs), namely transforming growth factor (TGF)-β and rapamycin, for 3–5 days. At the end of the incubation period, the iNKT cells were extensively washed and used to suppress the proliferation of alloreactive CFSE-labelled T cells.[7]

Upon culture with either rapamycin or TGF-β, the iNKT cells acquired the capability to inhibit the proliferation of alloreactive effector cells after stimulation with anti-CD3/CD28 (Figure 1). The inhibitory activity of the rapamycin- or TGF-β-treated iNKT cell lines was dose-dependent with a maximum inhibition detected at an iNKT:effector T-cell ratio of 1:1 and significant inhibition detectable at an iNKT:effector T-cell ratio of 1:3, although no inhibition was observed at a 1:10 iNKT:effector T-cell ratio.

Statistical analyses were performed, and data were calculated as the mean ± SD. For comparisons, ANOVA with the Kruskal-Wallis test was used.

Having established that iNKT cell lines treated with rapamycin or TGF-β can inhibit proliferation of alloreactive effector T cells, we performed a detailed phenotypic characterization of this inhibitory cell population, focusing on specific markers typically expressed by CD4+ Tregs, such as FOXP3, CTLA4, PDL1, HLA-DR, CD39 and CD62L. The iNKT cells did not express any of these markers, even after pretreatment with rapamycin or TGF-β (data not shown). Thus, it is conceivable that rapamycin- or TGF-β-pretreated inhibitory iNKT cells may use alternative regulatory mechanisms or mediate inhibition of T-cell proliferation by the production of suppressive cytokines, as previously reported.[5]

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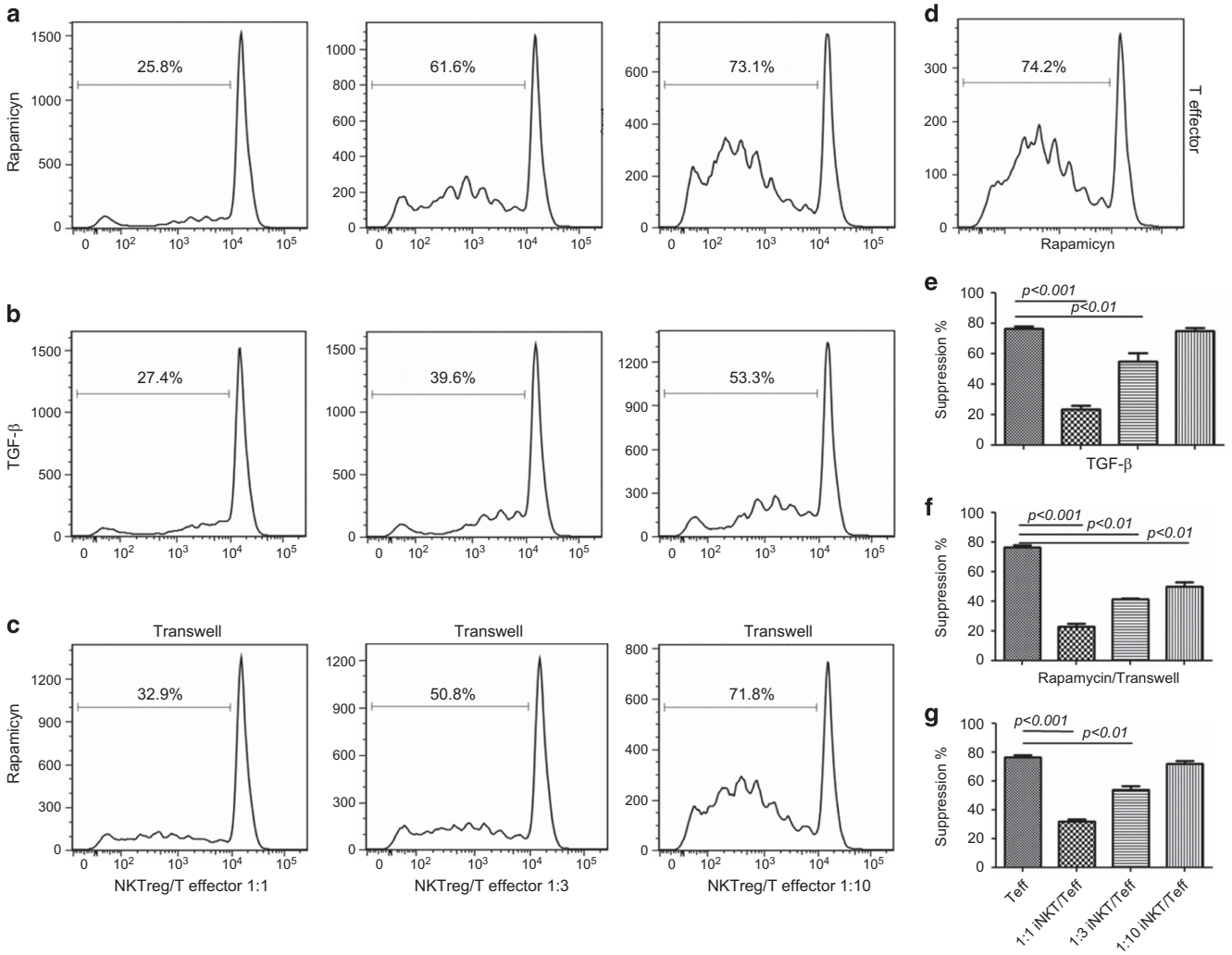

Figure 1 Suppressive activity of rapamycin- or TGF-β-treated human iNKTs. (**a**, **b**) Suppressive ability of rapamycin- or TGF-β-treated human iNKTs at various ratios of iNKT:effector T cell. (**c**) Suppressive ability of rapamycin-treated human iNKTs at various ratios of iNKT: effector T cell with a Transwell system. (**d**) Expansion of T effector cells. (**e**–**g**) The mean ± SEM of 10 independent experiments are expressed as the percentage inhibition of effector T-cell proliferation.

To gain insight into the mechanism by which regulatory iNKTs suppress T-cell proliferation, we performed Transwell experiments. The data obtained showed (Figure 1) that the regulatory iNKT cells were capable of inhibiting the proliferation of alloreactive effector T cells even when separated by a Transwell chamber. This result demonstrates that iNKT-mediated suppression does not require cell-to-cell contact. Further studies are thus required to determine the relative contributions of suppressive cytokines or other factors on the regulation carried out by regulatory iNKT cells.

Taken together, our preliminary data clearly indicate that peripheral blood-derived iNKT cells exposed to rapamycin or TGF-β rapidly acquire regulatory functions and are able to suppress the proliferation of T effector cells in a cell-to-cell-independent manner. These data further underline the regulatory function of iNKT cells and indicate the possibility of intentionally polarizing iNKT cell responses for therapeutic applications using drugs such as rapamycin that are commonly used in clinics.

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