Cytokine 53 (2011) 263–270

Contents lists available at ScienceDirect

Cytokine

journal homepage: www.elsevier.com/locate/issn/10434666

Review Article

Mechanism of regulation of autoimmunity by iNKT cells

Jan Novak [a,b,*], Agnes Lehuen [b,c]

[a] 3rd Faculty of Medicine, Charles University in Prague, Czech Republic
[b] INSERM U986, Hôpital Cochin-St. Vincent de Paul, Paris, France
[c] Université Paris Descartes, Paris, France

ARTICLE INFO

Article history:
Received 16 May 2010
Received in revised form 6 October 2010
Accepted 4 November 2010
Available online 23 December 2010

Keywords:
iNKT cells
Regulation
Autoimmunity
Cytokines
Cell contacts

ABSTRACT

iNKT cells, CD1d dependent natural killer T cells are a unique population of T cells. The capacity of iNKT cells to produce regulatory cytokines first provided an indication of their regulatory potential. Later on, in experimental models as well as in patients afflicted with an auto-immune disease, such as Type 1 diabetes mellitus, multiple sclerosis, and systemic lupus erythematosus along with others, a deficit in iNKT cell number was observed, suggesting the role these cells may possibly have in the prevention of auto-immune diseases. More importantly, experimental strategies which focused on increasing the volume or stimulation of iNKT cells in laboratory animals, demonstrated an improved level of protection against the development of auto-immune diseases. This article reviews the mechanism of protection against autoimmunity by iNKT cells, discusses the obstacles against and indications for the potential use of iNKT cell manipulation in the treatment of human auto-immune diseases.

1. Introduction

iNKT cells are CD1d dependent natural killer-like T cells. The term iNKT reflects one of the hallmarks of these cells, a co-expression of markers for both NK and T lineages. In addition to their expression of NK markers, iNKT cells exhibit several other phenotypical, developmental and functional characteristics that are not normally observed in conventional T cells.

First, iNKT cells are restricted by CD1d [1], MHC class I – like molecule that presents antigens of glycolipid and lipid structure in contrast to classical MHC molecules presenting peptides.

Second, contrarily to the infinite TCR repertoire of mainstream T cells, iNKT cells express a highly restricted TCR. Virtually all iNKT cells express the Vα14Jα18 chains in mice and Vα24Jα18 chains in human paired with restricted set of β chains – Vβ8.2, Vβ7, Vβ2 in mouse and Vβ11 in human respectively.

Finally, iNKT cells express activated/memory phenotype. Consistently with activated phenotype, iNKT cells exert rapidly effector functions without requirement for further activation. iNKT cells significantly influence the downstream network of immune cells, including NK cells, conventional T cells, Tregs, B cells, dendritic cells and others. Signaling between iNKT cells and downstream immune cells is mediated by either cell-to-cell contact (CD40-CD40L, OX40-OX40L interaction) and/or by cytokines. iNKT cells were shown to produce IL-2, IL-4, IL-5, IL-6, IL-10, IL-13, IL-17, IL-21, IFN-γ, TNF-α and GM–CSF (reviewed in [2–7]).

In addition to "classical" or "Type I" iNKT cells, other CD1d restricted T cells exist [5]. Type II NKT cells recognize hydrophobic antigens such as sulfatide [8] and their TCR repertoire is more diverse than that of iNKT cells [9]. Type II NKT cells were also shown to regulate autoimmunity [8,10] and tumor rejection [11,12], however their role in the regulation of immune responses is much less elucidated, therefore this review deals with classical (Type I) iNKT cells.

The discovery of the capacity of iNKT cells to produce regulatory cytokine IL-4 attached particular attention to these cells – they became considered potential regulators of the immune responses [13–15].

2. Regulation by iNKT cells

The hypothesis suggesting the regulatory potential of iNKT cells gained greater momentum, albeit indirectly, when it was found that there was a reduced number of iNKT cells in both patients

Abbreviations: iNKT, invariant natural killer-like T; NOD, non obese diabetic; TCR, T cell receptor; MHC, major histocompatibility complex; α-GalCer, α-galactosylceramide; IFN, interferon; TNF, tumor necrosis factor; TGF, transforming growth factor; GM–CSF, granulocyte–macrophage colony-stimulating factor; DN, double negative; EAE, experimental auto-immune encephalomyelitis; MS, multiple sclerosis; MOG, myelin oligodendrocyte glykoprotein; CNS, central nervous system; SLE, systemic lupus erythematosus; UC, ulcerative colitis; AIH, autoimmune hepatitis; ConA, concavalin A; RA, rheumatoid arthritis; MG, myasthenia gravis.

* Corresponding author. Present address: 3rd Faculty of Medicine, Charles University in Prague, Centre of Research for Diabetes, Endocrinological Diseases and Clinical Nutrition, Ruska 87, 100 00 Prague 10, Czech Republic. Tel.: +420 26716 3599; fax: +420 26716 3058.
E-mail address: novakjan@centrum.cz (J. Novak).

1043-4666/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.cyto.2010.11.001

afflicted with an auto-immune disease and experimental models of autoimmunity [16,17].

The most direct way to prove these hypotheses was to create a genetically engineered mouse, deficient in iNKT cells and to observe if it spontaneously developed an auto-immune syndrome. The experiments utilizing either CD1d$^{-/-}$ or Jα18$^{-/-}$ mice on a C57Bl/6 background however, proved unsuccessful. The iNKT deficient mice failed to develop spontaneous auto-immune disorders [16,18] contrarily to another population natural regulatory cells – CD4^{+}CD25^{+} FoxP3 – whose deficiency in FoxP3$^{-/-}$ lead to the spontaneous development of auto-immune diseases [19]. Several other observations however, display the regulatory potential of iNKT cells. Aging of C57BL/6 mice results in the lack of iNKT cells, and consequently, in the development of lupus like symptoms [20]. The introduction, of an "iNKT null" phenotype to a mouse that spontaneously developed an auto-immune disease such as the NOD (non obese diabetic) mouse (experimental model of type 1 diabetes mellitus) [21–23] or MRL-lpr mouse (experimental model of systemic lupus erythematosus) [24,25], had an impact on the course of autoimmunity in these animals, resulting in an increased incidence and severity of the disease. Furthermore, proof of the regulatory capacity of iNKT cells can be observed in experiments which prevent the development of auto-immune diseases in susceptible animals, by increasing the number of iNKT cells [26–28]. The data obtained during these experiments indicates that iNKT cells contribute to the complex process of the maintenance of immune tolerance.

In 1994, the pharmaceutical company Kirin discovered α-Galactosyceramide (α-GalCer) (a glycolipid derived from the marine sponge *Agelas Mauritianus*) and in 1997 Kawano demonstrated that this glycolipid specifically and effectively stimulated iNKT cells [29,30]. The discovery of α-GalCer provided an important instrument in tracking iNKT cells. α-GalCer loaded with tetramers of CD1d molecule (α-GalCer loaded CD1d tetramers) stain both mice and human iNKT cells with very high sensitivity and specificity [31,32]. The discovery of α-GalCer also allowed for further study of their biology and pathology. Several research groups utilized α-GalCer stimulation, in an attempt to enhance the regulatory potential of iNKT cells. Indeed, α-GalCer administration protected various experimental models of autoimmunity from development of disease [33]. Apart from its beneficial effect, α-GalCer induced various side effects including the accelerated autoimmunity in experimental models of multiple sclerosis, systemic lupus, colitis etc. [33,34].

The unpredictable outcome of α-GalCer administration in some experimental models of autoimmunity prompted the synthesis of new iNKT cell ligands. The manipulation of their truncated chain length allows for greater control of the cytokine secretion profile in iNKT cells, improving the safety profile and providing greater assurance in the beneficial outcome in autoimmunity [35–37].

2.1. Type 1 diabetes mellitus (T1DM)

The most extensive studies concerning the regulatory properties of iNKT cells were performed on the murine model of a prototypical auto-immune disease – type 1 diabetes mellitus. T1DM is a Th1 auto-immune disease caused by the destruction of pancreatic β-cells, responsible for insulin production [38–40]. The clinical understanding regarding the pathogenesis of T1DM has greatly benefited from the availability of a particular animal model – the NOD mouse. The NOD mice spontaneously develop T1DM which is clinically and immunologically similar to that of the human disease [41,42].

The initial observations linking iNKT cells with T1DM were independently provided by Gombert and Baxter, who during their studies noticed reduced numbers (up to 30%) of iNKT cells in NOD mice in comparison to non-auto-immune strains [26,43]. iNKT cells from NOD mice are also functionally deficient, producing low levels of IL-4 after TCR cross-linking and low levels of IFN-γ upon IL-12 stimulation [27,43,44]. The defect of iNKT cells in NOD mice has a genetic basis. The genome-wide screen identified two loci with significant linkage to iNKT cell numbers [45]. Nkt1 locus on distal chromosome 1 involves Slamf1 and Slamf6 genes which code for proteins involved in SAP signaling required for iNKT cell development [46], and Nkt2 locus on chromosome 2 which maps to the same region as the diabetes susceptibility gene Idd13 [45].

Further experiments demonstrated that the correction of iNKT cell defects greatly reduced the incidence of T1DM in NOD colonies. Two strategies to overcome these iNKT cell defects in NOD mice were published. Both, the over-expression of iNKT cells by genetic manipulation (transgenesis) [27] and the reconstitution of iNKT cells by cell transfer [26] provide lifelong protection. These experiments showed that the protection provided is "dose dependent" – the transgenic lines carrying the highest volume of iNKT cells, as well as mice transferred with the highest numbers of iNKT cells demonstrated the highest protection rates.

The initial research regarding the mechanism of iNKT cell mediated protection from T1DM amalgamated two main observations. First, Th1 deviation of the auto-immune response in NOD mice and the decreased ability of the NOD's immune system to develop a Th2 response [47,48]. Second, the potential of iNKT cells to produce IL-4, a potent inducer of the Th2 response [13–15]. The original hypothesis suggested that the reconstruction of iNKT cell numbers offers protection from T1DM by enhancing the secretion of IL-4 and thus salvages the Th2 response. Support of this hypothesis was observed in transgenic mice, which were indeed shown to produce higher levels of IL-4 in their peripheral lymphoid organs [49]. Furthermore, the level of protection from diabetes appeared to correlate to the levels of IL-4 in the peripheral lymph organs of transgenic mice [27]. This has long been believed to be yet another support for IL-4 dependence of iNKT cell mediated diabetes protection.

The polyclonal models used in these experiments however, made the distinction somewhat difficult, if the changes seen in iNKT transgenic mice (e.g. overproduction of IL-4) are indeed the mediators of diabetes protection. To better clarify this issue, Agnes Lehuen exploited a monoclonal model of diabetes originally described in Diane Mathis' laboratory [50]. In this model, the cloned CD4^{+} T-cells, originally isolated from the islets of a diabetic NOD mouse (BDC2.5 T cells), are transferred to lymphopenic recipients. This transfer leads to the rapid development of diabetes in 90–100% of T cell recipients. The presence of iNKT cells in recipients provides strong protection, with the incidence of diabetes being reduced to 10% [51].

The direct analysis of diabetogenic T cells transferred in iNKT cell harboring recipients revealed that the presence of iNKT cells inhibits the IL-2 and IFN-γ production and later proliferation of effector CD4^{+} T cell population. In addition to the defect of Th1 cytokine production (IL-2, IFN-γ and TNF-α) the production of Th2 cytokines (IL-4 and IL-10) by effectors is also significantly reduced. Thus, the effector CD4^{+} T cell population is found in hyporesponsive state – anergy – enabling them to destroy pancreatic β-cells [51].

Surprisingly, protection from diabetes is still maintained if NKT cells are unable to produce not only IL-4, but also if they fail to produce or induce other regulatory cytokines such as IL-10, IL-13 and transforming growth factor β (TGF-β). This data indicates that the regulatory cytokines are not key mediators in the natural regulation of T1DM by iNKT cells [52]. Cain and colleagues, however, suggest that the regulation of transfer-induced diabetes by iNKT cells is mediated by the IFN-γ [53]; cytokine, whose role was not tested