Cell Research (2015) 25:991-992.
© 2015 IBCB, SIBS, CAS All rights reserved 1001-0602/15 $ 32.00
www.nature.com/cr



# Copycat innate lymphoid cells dampen gut inflammation

*Cell Research* (2015) **25**:991-992. doi:10.1038/cr.2015.79; published online 26 June 2015

**The mechanisms whereby the gut mucosa tolerates trillions of commensal bacteria without developing inflammation remain poorly understood. A recent *Science* article reveals that gut innate lymphoid cells constrain inflammatory T cell responses to commensal bacteria by adopting a strategy usually deployed by thymic epithelial cells to negatively select self-reactive T cells.**

Innate lymphoid cells (ILCs) promote intestinal immunity by releasing distinct sets of cytokines that mirror those produced by $CD4^+$ T effector (Teff) cells [1]. Unlike ILCs, Teff cells express somatically recombined receptors that recognize antigen in the context of major histocompatibility class-II (MHC-II) molecules expressed on dendritic cells (DCs) [1]. The immunostimulating properties of Teff cells are constrained by $CD4^+$ T regulatory (Treg) cells expressing the transcription factor Foxp3 [2]. These Treg cells maintain gut homeostasis in cooperation with various cell types, including ILCs expressing the transcription factor RORγt and known as ILC3.

ILC3 maintain epithelial integrity and stimulate mucus secretion by releasing interleukin-22 (IL-22) and lymphotoxin [1, 3, 4], whereas Treg cells mitigate the pro-inflammatory activity of Teff cells by releasing transforming growth factor-β (TGF-β) and IL-10 [2]. Of note, these cytokines also stimulate B cell production of immunoglobulin A (IgA), a mucosal antibody class that controls commensal bacteria inhabiting the lumen of the gut [5].

Unlike ILC3, Treg cells require instructive signals from DCs to exert their homeostatic function. After capturing intraluminal antigen through transepithelial dendrites, $CX3CR1^+$ macrophages transfer antigen to $CD103^+$ DCs, which migrate to mesenteric lymph nodes (MLNs) to serve as antigen-presenting cells (APCs) for the generation of $Foxp3^+$ Treg cells [6, 7]. Besides MHC-II-restricted cognate T-DC interactions, this process involves release of TGF-β by DCs and conversion of dietary vitamin A into a tolerogenic metabolite known as retinoic acid (RA) in DCs [7].

Recent studies show that gut ILC3 induce Treg cells by releasing granulocyte monocyte-colony stimulating factor (GM-CSF) [8]. This cytokine elicits expression of RA in $CD103^+$ DCs in response to IL-1β from macrophages [8]. Besides inducing Treg cell expansion, ILC3 constrain Teff cell expansion by establishing MHC-II-dependent cognate interactions [9]. However, how do $MHC\text{-}II^+$ ILC3 exert this suppressive activity? An elegant study by Hepworth *et al*. [10] shows that $MHC\text{-}II^+$ ILC3 eliminate commensal-reactive $CD4^+$ Teff cells by adopting a negative selection strategy usually deployed by thymic epithelial cells (TECs) to eliminate self-reactive $CD4^+$ T cells.

Hepworth *et al*. [10] crossed mice expressing a TCR transgene specific for either the commensal antigen CBir1 or the food protein ovalbumin (OVA) with mice selectively lacking MHC-II in ILC3. In these mice, intestinal CBir1-specific but not OVA-specific Teff cells undergo spontaneous expansion, indicating that $MHC\text{-}II^+$ ILC3 regulate Teff cell responses to commensals under steady-state conditions [10]. Of note, mice selectively expressing MHC-II on ILC3 only show reduced expansion of adoptively transferred CBir1-specific Teff cells but not Treg cells when transgenic $CD4^+$ T cells are activated by CBir1 prior to adoptive transfer [10]. These findings indicate that $MHC\text{-}II^+$ ILC3 constrain Teff cells following their priming by commensal antigens (Figure 1). It remains to be verified whether $MHC\text{-}II^+$ ILC3 also regulate Teff cell responses to food antigens, including OVA.

Next, Hepworth *et al*. [10] determined that $MHC\text{-}II^+$ ILC3 inhabit the B-T border of MLNs, an area specialized in T cell priming by antigen. Considering that ILC3 crosstalk with $CD103^+$ DCs via GM-CSF, $MHC\text{-}II^+$ ILC3 may capture antigen from $CD103^+$ DCs in MLNs [8]. Alternatively, $MHC\text{-}II^+$ ILC3 may migrate to MLNs after acquiring antigen from $CX3CR1^+$ macrophages or $CD103^+$ DCs from the gut lamina propria [6]. Accordingly, ILC3 can home to mucosal draining lymph nodes by following chemotactive gradients established by CCR7 ligands [11].

Hepworth *et al*. [10] further found that MHC-II expression by ILC3 is uncoupled from expression of co-stimulatory molecules (Figure 1), even following activation by microbial or inflammatory stimuli. Studies from another group show that splenic ILC3 initiate productive $CD4^+$ T cell responses after upregulating both MHC-II and co-stimulatory molecules upon exposure to inflammatory stimuli [12]. One possibility that may reconcile these discrepant findings is that ILC3 inhibit co-stimulatory molecule expression in response to tissue-specific condition-

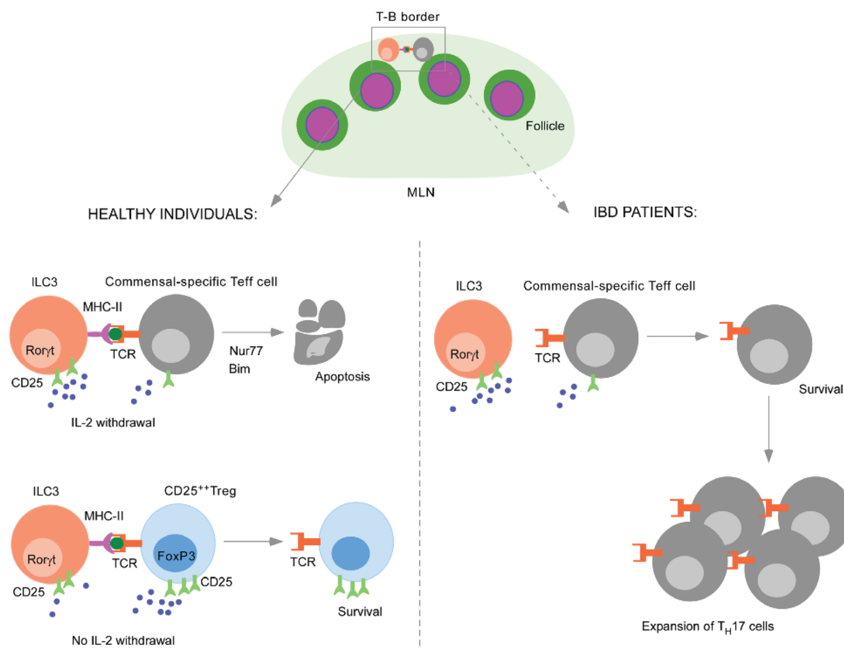


**Figure 1** ILC3 eliminate commensal-reactive CD4$^+$ T cells in healthy but not IBD individuals. Healthy subjects. MHC-II$^+$B7− ILC3 from T-B areas of MLNs establish MHC-II-restricted interactions that activate a Nur77-dependent Bim-mediated apoptotic program in commensal-specific CD4$^+$ Teff cells. In addition, MHC-II$^+$B7− ILC3 "starve" CD4$^+$ Teff cells by removing IL-2 from the extracellular environment through surface CD25. Unlike Teff cells, Treg cells express abundant surface CD25 and thus are resistant to IL-2 starvation. IBD patients. ILC3 fail to negatively select commensal-specific CD4$^+$ Teff cells due to defects of the molecular machinery supporting MHC-II expression. These defects induce expansion of Th17 cells.

ing signals emerging from gut but not splenic microenvironments.

Further transcriptional studies revealed that gut ILC3 express MHC-II through a mechanism involving the pIV promoter of class II transactivator (CIITA), a co-activator of MHC-II genes also used by TECs [10]. By setting up elegant *in vitro* ILC3-CD4$^+$ T cell co-cultures, Hepworth *et al*. [10] demonstrated that ILC3 activate a CD4$^+$ T cell death program similar to that activated by TECs for the negative selection of developing CD4$^+$ T cells. In the thymus, TECs eliminate autoreactive CD4$^+$ T cells by presenting self-antigen in the context of MHC-II. The resulting TCR signals activate a Nur77-controlled pathway that upregulates the pro-apoptotic protein Bim. A similar mechanism is also adopted by MHC-II$^+$ ILC3 to remove commensal-reactive Teff cells (Figure 1).

However, this is not all. Indeed, Hepworth *et al*. [10] found that MHC-II$^+$ ILC3 express elevated levels of CD25, a high-affinity receptor for the T cell growth factor IL-2. By extracting IL-2 from the extracellular environment via CD25, MHC-II$^+$ ILC3 starve CD4$^+$ Teff cells, thereby further restraining their expansion [10]. This finding may explain why MHC-II$^+$ ILC3 spare Treg cells (Figure 1), which express elevated levels of CD25.

Finally, Hepworth *et al*. [10] found that the gut ILC3 expressed less MHC-II in patients with inflammatory bowel disease (IBD). Concomitantly, these patients also showed more pro-inflammatory gut Teff cells, including T helper 17 (Th17) cells [10]. Thus, perturbations of MHC-II expression by ILC3 may cause gut inflammation by impairing the negative selection of CD4$^+$ Teff cells reactive to commensals (Figure 1). This raises the possibility that IBD may benefit from drugs capable to restore MHC-II expression by ILC3.

Giuliana Magri[1], Andrea Cerutti[1, 2, 3]

*[1]Institut Hospital del Mar 'Investigacions Mèdiques, [2]Catalan Institute for Research and Advanced Studies (ICREA), Barcelona Biomedical Research Park, Barcelona, Spain; [3]Immunology Institute, Department of Medicine, Division of Clinical Immunology, Icahn School of Medicine at Mount Sinai, New York, NY, USA*
Correspondence: Andrea Cerutti
Tel: +34-933-160-389; Fax: +34-933-160-410
E-mail: acerutti@imim.es or andrea.cerutti@mssm.edu

## References

1 Artis D, Spits H. *Nature* 2015; **517**:293-301.
2 Sakaguchi S, Vignali DA, Rudensky AY, *et al*. *Nat Rev Immunol* 2013; **13**:461-467.
3 Tumanov AV, Koroleva EP, Guo X, *et al*. *Cell Host Microbe* 2011; **10**:44-53.
4 Magri G, Miyajima M, Bascones S, *et al*. *Nat Immunol* 2014; **15**:354-364.
5 Kawamoto S, Maruya M, Kato LM, *et al*. *Immunity* 2014; **41**:152-165.
6 Mazzini E, Massimiliano L, Penna G, *et al*. *Immunity* 2014; **40**:248-261.
7 Coombes JL, Powrie F. *Nat Rev Immunol* 2008; **8**:435-446.
8 Mortha A, Chudnovskiy A, Hashimoto D, *et al*. *Science* 2014; **343**:1249288.
9 Hepworth MR, Monticelli LA, Fung TC, *et al*. *Nature* 2013; **498**:113-117.
10 Hepworth MR, Fung TC, Masur SH, *et al*. *Science* 2015; **348**:1031-1035.
11 Mackley EC, Houston S, Marriott CL, *et al*. *Nat Commun* 2015; **6**:5862.
12 von Burg N, Chappaz S, Baerenwaldt A, *et al*. *Proc Natl Acad Sci USA* 2014; **111**:12835-12840.