Research Highlights

Highlights from the latest articles in immunomodulation

Suppressor turns into helper

Evaluation of: Tsuji M, Komatsu N, Kawamoto S *et al.*: Preferential generation of follicular B helper T cells from Foxp3⁺ T cells in gut Peyer's patches. *Science* 323, 1488–1492 (2009).

One group of the suppressor T cells, regulatory T cells (Tregs), is important for understanding the immune responses and developing a new immunotherapy. It has been postulated that expression of the transcription factor Foxp3 specifically identifies the Treg lineage and acts as its 'master regulator. However, recent findings revealed that the Foxp3-positive T cells are more plastic than expected in their phenotype and function.

"The findings revealed that Treg cell differentiation upon alteration of Foxp3 expression is more dynamic than believed before."

In a recent paper published in *Science*, a research group led by Hori and Fagarasan reported that Foxp3⁺ T cells could convert to helper T cells, which support IgA production by B cells in the gut [1]. They transferred Foxp3⁺ or Foxp3⁻ T cells into T-cell-lacking mice. In the T-cell-lacking mice, because of the deficit of an efficient T-cell help, germinal center formation is not induced. Surprisingly, the authors found that formation of the germinal center in Peyer's patches was induced only in mice transferred with Foxp3⁺ T cells. Using serial transfer experiments, they also demonstrated that the transferred Foxp3⁺ T cells lost their expression and become effective precursors for follicular B-helper T cells that were capable of inducing germinal center formation in Peyer's patches. The Foxp3-downregulated T cells expressed costimulatory molecules such as CD40 ligand, inducible T-cell costimulator, or programmed death-1 that are necessary

for follicular B-helper T-cell generation and function. The Foxp3-downregulated T cells also expressed transcripts for IL-4 or IL-21 that contribute to germinal center formation. From these data, it was suggested that the environment of Peyer's patches has a capability to downregulate Foxp3 expression in Treg and convert their phenotype and functions. Furthermore, the differentiation of follicular B-helper T cells in Peyer's patches appeared to be controlled by the same signals that promote Foxp3 expression in gut T cells.

In humans, heterogeneity of Foxp3⁺ cells has recently been demonstrated. Miyara *et al.* reported that human Foxp3⁺CD4⁺ T cells were composed of three phenotypically and functionally distinct subpopulations: CD45RA⁺Foxp3^{lo} resting Treg cells and CD45RA⁺Foxp3^{hi} activated Treg cells, both of which were suppressive *in vitro*, and cytokine-secreting CD45RA⁺Foxp3^{lo} non-suppressive T cells. The resting Tregs could proliferate and convert into activated Tregs *in vitro* and *in vivo* [2].

Therefore, it seems that we must be more flexible when thinking about Tregs. The findings revealed that Treg cell differentiation upon alteration of Foxp3 expression is more dynamic than believed before. We must realize that Treg function and its Foxp3 expression are subject to change upon its circumstances, such as normal or disease states [3].

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Self-education by NK cells

Evaluation of: Chalifour A, Scarpellino L, Back J *et al.*: A role for *cis* interaction between the inhibitory Ly49A receptor and MHC class I for natural killer cell education. *Immunity* 30, 337 (2009).

The manner by which NK cells get into self-tolerance and acquire the competency to kill abnormal/nonself cells are very important questions remaining in the NK cell research field. NK cells are categorized as innate lymphocytes because they do not express antigen-specific receptors that are generated by gene recombination. However, NK cells can discriminate normal self and nonself/abnormal cells. To explain the self-tolerance in NK cells, many models have been proposed [1]. One of these, the 'missing self-recognition' theory [2] suggests that NK cells attack cells lacking self-MHC class I expression. However, NK cells in MHC class I-deficient mice are also tolerant to self cells, although they are less cytotoxic than those in normal mice. Kim *et al.* found that only NK cells that express inhibitory receptors for self-MHC class I can exert killing activity [3]. They

proposed a concept termed 'licensing' in which the inhibitory signals are essential for functional maturation of NK cells [3].

It has been known that there are two types of binding between NK cell receptors and their ligands: one is *in trans* (NK cells–other cells) and another is *in cis* (within one NK cell) [4]. The role for the *cis* binding of the inhibitory receptors has been unclear. Recently, Chalifour *et al.* developed mice that express variant Ly49A receptors that can bind to H2-D^d only *in trans* but not *in cis*. This variant Ly49A could inhibit NK cell effector functions, but did not induce an efficient NK cell maturation: namely, the killing activity of NK cells in the mice was clearly reduced [5]. Their data suggested that *cis* but not *trans* interaction of self H2-D^d and Ly49A on NK cells is important for the 'education' of NK cells.

Besides Ly49A, paired immunoglobulin-like receptors (PIRs) are known to be able to bind MHC class I *in cis*. It was reported that *cis* binding of PIR and MHC class I can regulate mast cell activation [6]. It was also reported that PIR is expressed in early stage of T-cell progenitors [7]. The *cis* interaction between inhibitory receptors and their ligands may be generally

involved in the functional maturation or differentiation of immune cells.

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Adjuvant effect of IL-7 in vaccine-mediated cancer immunotherapy

Evaluation of: Pellegrini M, Calzascia T, Elford AR *et al.*: Adjuvant IL-7 antagonizes multiple cellular and molecular inhibitory networks to enhance immunotherapies. *Nat. Med.* 15(5), 528–536 (2009).

IL-7 is a potent and pivotal cytokine for maintenance of lymphocyte homeostasis *in vivo* via supporting of B- and T-cell development and survival of both naive and memory T cells [1]. Its multiple immune-enhancing properties make it

a very attractive candidate for immunotherapy in wide variety of clinical situations, including tumor immunotherapy. However, it is hard to induce inflammation with IL-7 alone, therefore a combination of therapies may also be involved in inducing inflammation at eliciting an antitumor effect. Here, Pellegrini *et al.* reported that vaccine-induced antitumor response could be augmented by providing exogenous IL-7 via suppressing of molecular inhibitory networks in lymphocytes [2]. Transgenic mice expressing the SV40 large T antigen (TAG) and lymphocytic choriomeningitis

virus (LCMV) glycoprotein (GP) under the control of the rat insulin promoter (RIP) spontaneously develop pancreatic β -cell tumor and GPs containing LCMV infection can be used as vaccination for tumor antigen in this tumor model [3]. Treatment with IL-7 strongly improved LCMV-mediated vaccination against tumor. This improvement includes prolonged survival of RIP (GP $\times$ TAG2) mice, decreasing size of tumor, and enhancement of LCMV-specific T-cell effector function. Pellegrini *et al.* suggested that these enhancing antitumor effects following

IL-7 treatment might be dependent on upregulation of CD8⁺ T-cell-mediated cytotoxicity of tumors and increasing Th17, NK and NKT cell infiltration into the tumor-bearing pancreas [2].

How does IL-7 enhance the antitumor effect of lymphocytes and collaborate with vaccination? Pellegrini *et al.* also indicated the possibility that IL-7 induces Cbl-b reduction through Nedd4 upregulation [2]. Cbl-b is a RING-type E3 ubiquitin ligase and it can regulate cell signaling via ubiquitination of target substrate [4]. Previous reports indicated that Cbl-b increases the threshold for T-cell activation and contributes to anergy induction of lymphocytes [4]. Furthermore, genetic aberration of Cbl-b augments the activity of antitumor immune responses [5,6]. Similarity between IL-7-treated cells and Cbl-b-deficient cells was also highlighted. Therefore, IL-7-mediated Cbl-b downregulation is thought to be important to induce enhancement of the antitumor effect in this treatment.

Since IL-7-mediated support of vaccination is also observed in tumor antigen-pulsed dendritic cell injection and tumor antigen-expressing vaccinia virus immunization [2], it is conceivable that IL-7 treatment can be used for a wide-variety of vaccine-based tumor immunotherapies. These findings strongly suggest that addition of IL-7

along with vaccination can substantially increase antitumor immunity, and IL-7 treatment may have an adjuvant role in clinical application for vaccine-mediated immunotherapy against tumor antigens in cancer patients.

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“...addition of IL-7 along with vaccination can substantially increase antitumor immunity, and IL-7 treatment may have an adjuvant role in clinical application for vaccine-mediated immunotherapy against tumor antigens in cancer patients.”