

INTERVIEW about

CTLA-4 and GATA-3
with

Claudio Pioli, Ph.D., ENEA (Italian agency for new technologies, energy and environment) Section of Toxicology and Biomedicine Rome, Italy

pioli@casaccia.enea.it



What is known about GATA3?

Claudio Pioli: GATA3 is a factor involved in several differentiating processes including nervous system development in embryo and T-cell lineage commitment. Involvement in cell lineage specification is a common feature of the GATA-binding transcription factor family, which includes five other members besides GATA-3. In mice, as GATA-3 gene targeting leads to embryonic lethality, studies on T-cell specific GATA-3 function were performed through RAG blastocyst reconstitution or lineage-specific inducible gene-targeting. These studies revealed that GATA-3 plays a relevant role

during thymic T cell differentiation at several stages. GATA3 activity, indeed, is required by the earliest T cell progenitors (DN1, CD44⁺CD25⁺CD4⁺CD8⁻ cells) to progress to the following stage. Later in differentiation, up-regulation of GATA3 expression in double positive (DP, CD4⁺CD8⁺) thymocytes is associated with differentiation to single positive (SP) CD4 cells whereas its down-modulation leads to SP CD8 cell commitment. But GATA-3 continues to affect T cell fate also in the periphery, by promoting mature naive CD4 T cell differentiation to T-helper 2 (Th2) effector cells.

What is the role of GATA3 in CD4 T helper 2 cell differentiation?

Claudio Pioli: Upon activation, naive CD4 T cells begin a differentiation process that commits these cells to produce a specific pattern of cytokines. T helper 1 (Th1) cells produce IFN γ and promote cell-mediated immune responses against intracellular pathogens. Th2 cells produce IL-4, IL-5 and IL-13, sustain immune responses against parasites such as helminths, and favor the onset of allergies. Protective immunity depends on the proper balance between Th1 and Th2 cell responses. In this context, GATA3 is considered a leading factor for Th2 cell differentiation (1,2). GATA-3 over-expression, indeed, leads to the production of Th2-type cytokines even in Th1-dif-

ferentiated cells. Under physiological conditions, GATA3 is expressed at low level in naive CD4⁺ T cells and increased during Th2 cell differentiation. Antigen recognition by T-cell receptor (TCR) and CD28 costimulation promote GATA3 expression. But the most powerful stimulus that sustains GATA-3 mRNA expression is IL-4. This Th2 type cytokine, indeed, promotes Th2 cell differentiation through the activation of STAT6, a potent transactivator for *gata3* gene. When GATA3 concentration exceeds a certain threshold, which is modulated by inhibitory proteins, it is able to promote its own expression and stabilize Th2 differentiation.

How does GATA3 induce Th2 cell differentiation?

Claudio Pioli: GATA-3 can act as transactivator for several genes, including *maf* (a transcription factor that selectively transactivates *Il-4*), *Il-5* and, as already said, *gata3* itself. However, its major role is to establish tran-

scriptional competence for the Th2 cytokine gene cluster (*Il-13/Il-4/Il-5*) through chromatin remodeling. Antigen-stimulated naive CD4⁺ T cells show non-selective transcription of Th1 and Th2 cytokine genes. During

differentiation one set of genes is epigenetically activated and the other one is silenced. GATA3-induced changes in chromatin structure lead to derepression, acquisition of DNase hypersensitivity and transcription of Th2 cytokine genes. GATA3, by binding to several specific sites in Th2 gene cluster, allows displacement of MBD2. This methyl-CpG binding domain protein, together

with other proteins, recruits corepressors and histone deacetylases leading to a inaccessible chromatin structure. MBD2 displacement seems to occur at the time of transcriptional induction and before DNA demethylation. Later during differentiation, the *Il4* locus undergoes progressive demethylation in Th2 cells, this latter process being not a direct effect of GATA-3. (1,2)

Has CTLA-4 an influence on GATA-3 expression?

Claudio Pioli: The engagement of the negative costimulatory receptor CTLA-4 (CD152) during cell polarization inhibits Th2 but not Th1 cell differentiation (3). This finding is also supported by the observation that CTLA-4 knock out mice have CD4 T cells with a predominant Th2 type cytokine pattern and high levels of serum IgE (4). We found that CTLA-4 stimulation inhibits GATA-3 mRNA expression (5).

At variance, it does not affect the expression of T-bet, the Th1-leading differentiating factor. T-bet negatively regulates Th2 cell differentiation by binding and sequestering GATA-3 (6), pointing to the importance of GATA-3/T-bet stoichiometry within the differentiating Th cell. Thus, CTLA-4, by reducing GATA-3/T-bet ratio, favors the sequestering action of T-bet, and negatively controls Th2 cell differentiation.

Why do you believe that CTLA-4 has an effect on STAT6 activation?

Claudio Pioli: We found that GATA-3 mRNA expression is inhibited when naive CD4 T cells are stimulated under both neutral (i.e. absence of cytokines) and Th2-polarizing (i.e. presence of IL-4) conditions. In fact, under Th2-polarizing conditions, the inhibitory effect of CTLA-4 on GATA-3 expression is larger than under neutral conditions. This finding suggested us that CTLA-4 might affect not only TCR/CD28-induced signals

but also IL-4-mediated effects on GATA3 expression. As we already discussed, IL-4 activates STAT6 which in turn promotes GATA3 expression. We found that CTLA-4 engagement inhibits STAT6 activation. STAT6 activation (dimerization and nuclear transfer) depends on tyrosine phosphorylation. Our results demonstrate that CTLA-4 engagement during Th cell differentiation leads to a reduced STAT6 phosphorylation (5).

How does CTLA-4 affect STAT6 phosphorylation?

Claudio Pioli: CTLA-4-associated phosphatases have been involved in its negative signals as well as inhibition of phosphatase activity may result in STAT6 activation. However, we did not find evidence for a direct effect of CTLA-4 on STAT6 activation. Our idea is that CTLA-4 engagement during Th cell differentiation renders cells less responsive to IL-4 stimulation. STAT6 activation depends on the heterodimerization of IL-4R α chain and common γ chain as induced by IL-4 binding. Upon activation, naive CD4 T cells up-reg-

ulate IL-4 α chain expression. Our data show that CTLA-4 engagement results in a compromised IL-4R α chain expression (5). As a consequence, cells are less responsive to IL-4, display reduced STAT6 activation and do not up-regulate GATA-3 expression.

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