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Anne Dejean, Emeline Joulia, Thierry Walzer. The role of Eomes in human CD4 T cell differentiation: A question of context. *European Journal of Immunology*, 2019, 49 (1), pp.38-41. 10.1002/eji.201848000 . hal-03584989

HAL Id: hal-03584989

<https://ut3-toulouseinp.hal.science/hal-03584989>

Submitted on 22 Feb 2022

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COMMENTARY

The role of Eomes in human CD4 T cell differentiation:
A question of contextAnne S. Dejean¹, Emeline Joulia¹ and Thierry Walzer² ¹ UMR Inserm, U1043, Toulouse 31300, France; UMR CNRS, U5282, Toulouse 31300, France, Université de Toulouse, UPS, Centre de Physiopathologie de Toulouse Purpan (CPTP), Toulouse, France² CIRI, Centre International de Recherche en Infectiologie - International Center for Infectiology Research, Inserm, U1111, Ecole Normale Supérieure de Lyon, Université Lyon 1, CNRS, UMR5308, Lyon, France

Eomesodermin (Eomes) is a transcription factor (TF) of the T-box family closely related to T-bet known for its role in CD8 T cell and natural killer cell differentiation. However, the role of Eomes in CD4 T-cell differentiation is less well appreciated. In this issue of the *European Journal of Immunology* [Eur. J. Immunol. 2019. 49: 79–95] Mazzoni et al. and [Eur. J. Immunol. 2019. 49: 96–111] Gruarin et al. studied the role of Eomes in human CD4 T-cell differentiation. Mazzoni et al. showed that Eomes plays a key role in helper T cell (Th) plasticity by favoring the phenotype shift of Th17 cells toward non-classic Th1 cells; while Gruarin et al. proposed Eomes as a lineage-defining TF for human IL-10 and IFN- γ co-producing regulatory T-cells (Tr1 cells). Both studies show that Eomes drives IFN- γ secretion and stamps a “cytotoxic” signature, while it also represses Th17 features. However, additional signals including the cytokine milieu may further influence the fate of Eomes⁺ CD4 T cells. A common feature of Eomes⁺ CD4 T cells appears to be their accumulation in inflamed tissues in patients with chronic inflammatory disorders. Whether Eomes favors expression of the proinflammatory cytokines or on the contrary, promotes the anti-inflammatory cytokines, remains a matter of debate.

Keywords: EOMES · CD4 T cells · Regulatory T cells · Th1 · Th17

See accompanying articles by Mazzoni et al. and Gruarin et al.

CD4 T cells can adopt different functional fates depending on the set of transcription factors (TF) they express, these TF being induced by various polarizing cytokines. Th1, Th2 and Th17 helper lineages are defined by expression of T-bet, Gata-3 and ROR γ t expression, respectively. Helper CD4 T cell subsets are also known to be highly plastic, especially the Th17 type. Th1 cells can derive directly from naïve CD4 T cells or from differentiated Th17 cells.

Mazzoni et al. have defined the cells from the latter scenario as “non-classic” Th1 cells [1]. Non-classic Th1 cells can also be identified in vivo, further substantiating the idea that plasticity is a relevant feature of Th cells. However, the TF that promote non-classic Th1 differentiation from Th17 precursors and stabilize their lineage have not been defined.

Regulatory T cells (Tregs) are marked by Foxp3 expression, which can be acquired during development in the thymus. An additional regulatory T cell type, distinct from Tregs has been identified more than 20 years ago in vitro. The so-called type 1

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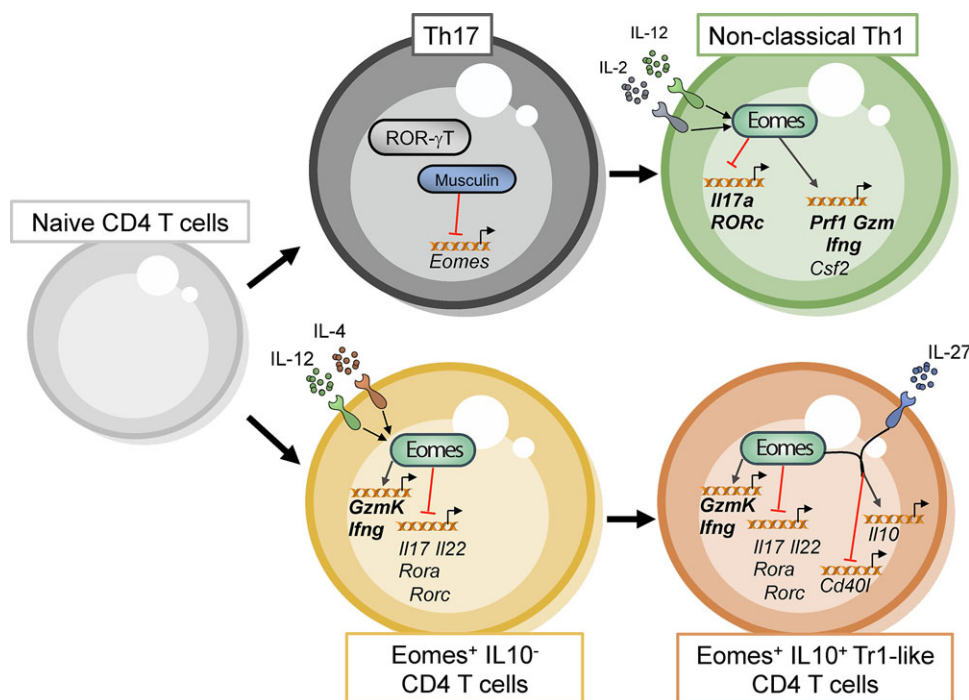


Figure 1. Potential role of Eomes in human CD4 T cells: In human CD4 T cells, Eomes drives the expression of IFN- γ and cytotoxic molecules (*Prf1* and *Gzm* genes), while inhibiting human Th17 differentiation. Mazzoni et al. propose that Eomes drives the phenotype shift of Th17 cells toward non-classic Th1 cells [1], while Gruarin et al. propose Eomes as a lineage-defining TF for human Tr1 cells co-producing IFN- γ and IL-10 in response to IL-27 signaling (genes in bold are genes for which direct regulation of Eomes has been demonstrated in human CD4 T cells) [3].

regulatory T cells (Tr1) are defined by their IL-10 production and are capable to suppress T cell responses in vivo [2]. Although the conditions of IL-10 production by T cells in several diseases have been progressively deciphered over the years, the identification of the Tr1 lineage in vivo has remained problematic. Several markers of Tr1 have been proposed but none of them is really specific. An important unresolved question is whether Tr1 cells form a stable lineage with defining features or not. Gruarin et al. have previously reported that IL-10/IFN- γ co-producing Tr1-like cells can be identified in the CD4⁺CD25-IL7R-CCR5+PD1+ fraction, which was a framework to study these cells in more details [3].

Using gene expression profiling strategies, both Mazzoni et al. and Gruarin et al. report that a defining feature of both non-classic human Th1 and Tr1 cells is their expression of the TF Eomes. Eomes is one of the two T-box TF expressed in the immune system. It is highly expressed by NK cells and various subsets of effector and memory CD8 T cells. In these immune subtypes, Eomes is thought to be essential to drive responsiveness to IL-15 by promoting the expression of CD122 [4]. Moreover, in CD8 T cells and ILCs, Eomes promotes the acquisition of cytotoxic attributes [5, 6]. Deletion of Eomes and T-bet in CD8 T cells also lead to a de-repression of Th17 genes [7].

In CD8 T cells prolonged TCR stimulation, combined with IL-2 induce high Eomes expression [8]. However, the conditions leading to Eomes induction in CD4 T cells may depend on their differentiation stage. Mazzoni et al. reported that IL-2 and IL-12 cooperate for Eomes transcription initiation in Th17 clones [1]. Gruarin et al. performed a more comprehensive analysis of the

conditions that lead to Eomes induction in naïve CD4 T cells [3]. The authors confirmed that IL-4 was sufficient to induce Eomes in CD4 T cells stimulated through their TCR, as previously demonstrated for CD8 T cells [9]. This group was able to further show that stimulation of naïve CD4 T cells with mature DC expressing IL-12 and with an exogenous supply of IL-4 led to the induction of Eomes⁺ IFN- γ + GZMK⁺ cells, which could be precursors of Tr1 cells. However, this cocktail was not sufficient to induce IL-10 production, and thus induce Tr1 cell differentiation, while IL-27 was found to be a critical factor, at least when Eomes was expressed using lentiviral vectors in human CD4 T cells. A previous study had identified Eomes as an important TF for the differentiation of mouse Tr1 cells [10]. In this study, Eomes expression in Tr1 cells was found to be downstream of IL-27 and Blimp-1. Thus, the cytokines responsible for Eomes expression in CD4 T cells both in mice or human remains a matter of debate and may depend on the differentiation status of CD4 T cells.

Both Mazzoni et al. and Gruarin et al. demonstrated that induction of Eomes via lentiviral vectors in Th17 clones or in naïve CD4 T cells stimulated through their TCR had three important consequences (Fig. 1). (i) Eomes induced high IFN- γ expression. This is in agreement with Yang et al. who showed that Eomes induction could rescue IFN- γ production by T-bet deficient T cells [11]. Gruarin et al. showed that Eomes could directly bind IFN- γ promoter, thus confirming previous studies in mouse [11, 12]. (ii) Eomes promoted the acquisition of cytotoxic features, such as the expression of *Prf1* and various granzymes, and the capacity to degranulate upon stimulation. Eomes overexpression induced an

increase of both GzmK and CD107a. These cytotoxic functions of Eomes⁺ CD4 T cells were previously described in MS patients as well as in the mouse model of EAE [13] or in RA patients [14]. Gruarin et al. addressed the question of the relationship between cytotoxic CD4 T cells (CTL) and Tr1 cells, which both express Eomes and are therefore difficult to separate from each other. The authors assume that CD4 CTL are CD27⁻, based on a previous demonstration that human CD8⁺CD27⁻ are more cytotoxic than their CD27⁺ counterpart, and propose that Tr1 cells are CD27⁺, which is supported by higher IL-10 production in this subset compared to CD27⁻ cells. Yet, this dichotomy is not perfectly clear and the production of IL-10 by the CD27⁺ subset is actually highly variable between individuals, which questions the stability of the Tr1 lineage, or the relevance of the CD27 marker. (iii) Eomes represses Th17 features, such as IL17 secretion, RORC2 and IL17A transcription [1], CCR6, IL-17, RORC and RORA expression [3]. Moreover, Eomes can directly bind RORC2 and IL17A [1]. This is again in agreement with results obtained in murine studies: Eomes binds to and inhibits *Rorc* and *Il17* promoter activation [15].

Interestingly, there were some discrepancies between both papers related with the expression of IL-10 and GM-CSF by Eomes⁺ CD4 T cells. First, Geginat and colleagues proposed Eomes as a lineage-defining TF for human IL-10 producing Tr1 cells but, as discussed previously, Eomes expression is probably not sufficient to drive IL-10 expression. Regarding GM-CSF, Annunziato and colleagues demonstrate that Eomes expression is associated with a highly inflammatory phenotype in patients with juvenile idiopathic arthritis (JIA) [1]. These results are in agreement with previous studies in mice [12] but also human CD4 T cells [13], which associated Eomes expression with pathogenic inflammatory functions and IFN- γ /GM-CSF secretion. However, Geginat and colleagues showed, using genome-wide gene expression analysis, that CSF2 is “among the most downregulated genes » (as IL-17F, IL-22 or ROR α) in Eomes⁺ Tr1-like cells. The Th17 origin of non-classic Th1 cells may therefore allow them to maintain GM-CSF production, even if Eomes is expressed, which may not be the case for precursors of Tr1 cells.

A common feature of Eomes⁺ CD4 T cells appears to be their accumulation in inflamed tissues in patients with inflammatory disorders. Mazzoni et al. reported an enrichment of Eomes⁺ IFN- γ + and IFN- γ +GM-CSF+ cells in the synovial fluid of JIA patients compared to the peripheral blood [1]. Eomes⁺ CD4⁺ T cells were also increased in the peripheral blood and cerebrospinal fluid from patients in a progressive state of multiple sclerosis [13] and in the synovial fluid of RA patients [14]. Moreover, Gruarin et al. observed that Eomes⁺Tr1-like cells were strongly enriched in the inflamed gut of IBD patients as compared to peripheral blood. Further studies will be needed in mouse models to understand what drives the accumulation of these Eomes⁺ CD4 T cells in inflamed tissues, with regard to cytokines that may induce Eomes, and trafficking/retention mechanisms that could lead to their accumulation in such tissues.

In conclusion, Eomes is unlikely defining a human CD4 T cell lineage. This is supported with the following: (i) Eomes expression can be found in nearly all Th subtypes both in mice and human. (ii)

The cytokines and signaling pathways leading to Eomes expression in CD4 T cells are still not well defined. (iii) The specific function of Eomes remains a matter of debate: Eomes was associated with inflammatory cytokine secretion and pathogenic populations in several studies [12–14]; while in others, it drives the differentiation of Tr1 cells producing IL-10 which has regulatory role in GvHD [10]. The question of the stability versus plasticity of the Eomes⁺ lineage is not fully addressed. In particular, CD4 CTL and CD4 Tr1 are highly similar in many aspects and there could be interconversion between these subsets, IL-27 and perhaps other cytokines being instrumental in this phenomenon. The only consensus so far is that Eomes⁺ CD4 T cells might play a role in chronic inflammatory disorders where their frequency is highly increased, suggesting that Eomes drives the expression of genes involved in proliferation or inflammation. In-depth analysis of the molecular function of this TF and identification of direct target genes will be needed to clarify its role in CD4 T cells.

Acknowledgments: This work was supported financially by grant by the The Agence Nationale de la Recherche (ANR; project GAMBLER)

Conflict of interest: The authors declare no financial or commercial conflict of interest.

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Received: 30/11/2018
Revised: 30/11/2018
Accepted: 6/12/2018