HMG-CoA reductase inhibitor and lowers sterol precursor levels in Insig-DKO embryos. This experiment strongly supports the idea that elevated sterol precursors underlie the facial clefting. Future work will investigate whether this is mediated through an alteration of Shh signaling.

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T lymphocytes originate from the thymus. Evidence has been accumulated to demonstrate that “natural” Tregs, whose major function is to control autoimmune responses, stem as a separate lineage in the thymus. These natural Tregs are CD4\(^+\); they express the transcription factor Foxp3, which represents a lineage marker, and high levels of CD25. Natural Tregs that migrate to the periphery keep their phenotypic and functional properties, which are essentially cytokine independent. In parallel, other subsets of CD4\(^+\) Tregs have been described that very effectively control immune responses not only to self antigens but also to a wide variety of nonself antigens (microbial, tumoral, and transplantation antigens). These Tregs are not present as such in the thymus; they derive from peripheral precursors that are CD4\(^+\)-CD25\(^-\)-Foxp3\(^-\) and differentiate into functional Tregs following adequate stimulation (in the presence of the cognate antigen and specialized immunoregulatory cytokines, i.e., TGF-\(\beta\), IL-10, and IL-4). They are generally termed “adaptive” Tregs. Once differentiated, adaptive Tregs, like natural Tregs, may express CD25 and Foxp3. However, one main feature that differentiates adaptive from natural Tregs is their unique cytokine dependence. The study by Vukmanovic-Stejic et al. in this issue of the JCI (14) proposes that, in humans, even at a very advanced age, adaptive Tregs essentially emerge from CD4-CD25\(^-\) T cells belonging to the memory T cell pool (previously primed by cognate antigens). Presently, one cannot exclude, however, that especially in young adults, some adaptive Tregs may emerge from naive, peripheral CD4-CD25\(^-\) T cells.

**Figure 1**

T lymphocytes originate from the thymus. Evidence has been accumulated to demonstrate that “natural” Tregs, whose major function is to control autoimmune responses, stem as a separate lineage in the thymus. These natural Tregs are CD4\(^+\); they express the transcription factor Foxp3, which represents a lineage marker, and high levels of CD25. Natural Tregs that migrate to the periphery keep their phenotypic and functional properties, which are essentially cytokine independent. In parallel, other subsets of CD4\(^+\) Tregs have been described that very effectively control immune responses not only to self antigens but also to a wide variety of nonself antigens (microbial, tumoral, and transplantation antigens). These Tregs are not present as such in the thymus; they derive from peripheral precursors that are CD4\(^+\)-CD25\(^-\)-Foxp3\(^-\) and differentiate into functional Tregs following adequate stimulation (in the presence of the cognate antigen and specialized immunoregulatory cytokines, i.e., TGF-\(\beta\), IL-10, and IL-4). They are generally termed “adaptive” Tregs. Once differentiated, adaptive Tregs, like natural Tregs, may express CD25 and Foxp3. However, one main feature that differentiates adaptive from natural Tregs is their unique cytokine dependence. The study by Vukmanovic-Stejic et al. in this issue of the JCI (14) proposes that, in humans, even at a very advanced age, adaptive Tregs essentially emerge from CD4-CD25\(^-\) T cells belonging to the memory T cell pool (previously primed by cognate antigens). Presently, one cannot exclude, however, that especially in young adults, some adaptive Tregs may emerge from naive, peripheral CD4-CD25\(^-\) T cells.

**Characteristics of adaptive Tregs in humans**

It is this complex, though highly relevant, problem that Akbar’s group addresses in their study in this issue of the JCI (14). Akbar, Vukmanovic-Stejic, and colleagues present data showing that the proportion and functional integrity of CD4\(^+\)CD25\(^-\)Foxp3\(^+\) Tregs are maintained in older (>70 years) human subjects and that these cells most probably do not derive from the thymic lineage of CD4\(^+\)CD25\(^-\)Foxp3\(^+\) Tregs.

The authors used an original method based on the in vivo incorporation of deuterium-labeled glucose (or glucose derivatives) into the DNA of dividing cells, with ultimate follow-up of the labeled population and subsequent evaluation of cell replication and survival within phenotypically distinct peripheral T cell subsets (14). The results obtained convincingly show that throughout the individual’s whole life span, lymphocytes corresponding to the phenotypic and in vitro functional definition of Tregs are generated from the peripheral pool of CD4\(^+\)CD45RO\(^-\)CD25\(^-\)Foxp3\(^-\) memory T cells (Figure 1).

The distinction between “natural” Tregs of thymic origin and “adaptive” Tregs exclusively generated from peripheral CD25\(^-\) lymphocytes following adequate conditions (including antigen stimulation and cytokine milieu) was suggested a few years ago from data obtained and validated in the mouse. It was then shown that peripheral CD25\(^-\) T cells whose T cell receptor or coreceptors (e.g., CD4) are stimulated in the presence of TGF-\(\beta\) acquire regulatory properties that may be assessed both in vitro and in vivo (2, 4, 15, 16).

The results reported here by Vukmanovic-Stejic et al. (14) may represent the first indication of a similar dichotomy in human Tregs. If confirmed, 2 other sets of data are of particular interest, namely those showing that human Tregs have a shorter doubling time when compared with other subsets studied (e.g., peripheral classical memory and naive-type T cells) and that they appear particularly sensitive to apoptosis. Such observations may present a solid argument for the dependence of human adaptive Tregs on continuous antigen stimulation and/or the presence of growth factors in their immediate environment for their differentiation and survival. This could turn out to be an essential condition of their homeostasis.

**Pending issues**

These conclusions should be tempered, however, by an appreciation of the technical limitations of investigations performed in humans. The first concern is that the rationale of the work and its interpretations are based on the assumption that a high level of CD25 expression is a reliable marker for human Tregs. It is unquestionable that the CD4\(^+\)CD25\(^-\) T cell subset concentrates both the majority of Foxp3\(^+\) cells and the essential part of functional regulatory capacity as assessed by in vitro coculture. However, how sure are we that these in vitro functional assays in humans reflect an in vivo regulatory functional capacity as shown in the mouse, using adoptive transfer experiments? Moreover, recent data suggest that the IL-7 receptor (CD127) is downregulated on a subset of human peripheral CD4\(^+\) T cells that are...
Foxp3+ and suppressive but that express no or low levels of CD25 (17). Additionally, one may consider whether Foxp3 in the human as opposed to the mouse is also expressed by activated T cells, independently of any regulatory function.

The second concern, closely linked to the first, is the critical issue of the antigen specificity of the Tregs studied, which is only very indirectly addressed (14). The results showing a biased T cell repertoire restricted to a given VF family (VF2 in this case) in a representative individual with persistent CMV infection are intriguing. However, the regulatory functional capacity of the CD4+CD25–Foxp3+ cell subset detected in thisVF2 anti-CMV population remains to be demonstrated.

To conclude, it appears plausible to extend to the human the dichotomy proposed in the mouse that distinguishes natural versus adaptive Tregs, which have distinct origins, namely, thymic-derived CD4+CD25–Foxp3+ cells in the case of natural regulatory lymphocytes versus peripheral memory-type CD4+CD25– precursors in the case of adaptive Tregs. In this context, it will be important to further experimentally dissect the adaptive Treg subset to more directly address whether or not the differences that have been proposed for each subset (e.g., Th2, Th3, Tr1, CD45RBlow T cells) in terms of phenotype and putative cytokine dependency are indeed a reflection of their belonging to distinct cell lineages.

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You say estren, I say estrogen.
Let’s call the whole replacement off!

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Estrogens and androgens play a key role in regulating bone mass. However, their clinical use as bone anabolic agents is limited due to unwanted side effects, particularly in reproductive organs. In 2002, the synthetic ligand estren was described to reproduce the bone anabolic, nongenotropic effects of sex steroids while having no effect on the uterus or seminal vesicles. But in the current issue of the JCI, Windahl et al. provide data showing that estrens are not as suitable a replacement for estrogen as was initially reported (see the related article beginning on page 2500). Though not catabolic, estrens triggered only minor, nonsignificant increases in bone mass in gonadectomized mice, all while inducing hypertrophy of reproductive organs. Does this mean estrens should not be pursued as a therapy for osteoporosis?

Estrogen and its receptors

The estrogen hormone family plays an essential role in the regulation of skeletal growth and homeostasis. While osteoblasts, osteocytes, and osteoclasts can be indirect targets of hormone signaling, they are also direct targets of estrogen and express functional estrogen and androgen receptors (ER and AR, respectively) (1). As estrogen or androgen deficiency can lead to rapid decreases in bone mass, therapies designed to return these sex hormones to their original levels would seem logical. However, these strategies have been fraught with difficulty due to the complex nature of hormone signaling.

In the classical (genomic) model of estrogen signaling, estrogens bind to the ER in the nucleus (Figure 1). Over the course of several hours, the estrogen-ER complex then induces a direct response through estrogen response element sequences or an indirect response by triggering expression of other proteins such as transcription factors of the AP1 family, among others. This is viewed as the main

Nonstandard abbreviations used: AR, androgen receptor; ER, estrogen receptor; SERM, selective estrogen receptor modulator.

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