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The renaissance of T regulatory cells: Looking for markers in a haystack

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EL RENACIMIENTO DE LAS CÉLULAS T REGULADORAS: BUSCANDO MARCADORES EN UN PAJAR

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RESUMEN

Un tema básico en la Inmunología es cómo el Sistema Inmunitario puede proteger al huésped frente a una extraordinaria variedad de organismos patógenos al mismo tiempo que controla esas respuestas para que su duración o intensidad no sean perjudiciales para el organismo. Desde hace varios años se han acumulado los datos que subrayan la importancia de linfocitos T diferenciados en el timo, denominados linfocitos T reguladores (Treg), en la supresión de las respuestas inmunitarias normales y patológicas, contribuyendo a la tolerancia a los elementos propios y a la homeostasis inmune. Su papel en el control de la respuesta inmunitaria frente a tumores, alergenos, patógenos e injertos alogeneicos ha llamado la atención hacia su potencial uso terapéutico. Sin embargo, para que este potencial pueda convertirse en realidad es precisa una buena caracterización fenotípica y funcional de esta subpoblación, una tarea que se ha comprobado dificultosa. Así, todavía no están claros muchos puntos acerca de los genes diferenciadores maestros de este linaje celular, sus marcadores de superficie específicos, o sus mecanismos de supresión.

En dos trabajos muy recientes se han descrito las nucleotidasas CD39 y CD73 como marcadores de superficie de las células Treg, lo que permite unir la actividad supresora de estas células con modelos previos de inmunosupresión en los cuales la adenosina y el AMP cíclico tenían un papel funcional primordial.

PALABRAS CLAVE: Linfocitos T reguladores/ Treg/ /Nucleotidasas/ CD39/ CD37 /Adenosina/ cAMP.

ABSTRACT

A key issue in Immunology is how the Immune System manages to achieve its major aim of protecting the host against an extraordinary variety of pathogens while, at the same time, controlling responses whose perduration and intensity might be harmful to the organism. For some years now, evidence has come out of the importance of a thymus-derived T cell subpopulation, called "regulatory" (Treg), able of suppressing physiological and pathological responses, contributing to self tolerance and immune homeostasis. Its role in controlling immune response to tumours, allergens, pathogens and allogeneic grafts has driven the attention towards its therapeutic potential. However, to develop this therapeutic potential to the full a good phenotypical and functional characterization of this subpopulation is necessary, a task that has proven difficult. Thus, many factors still remain obscure concerning the master differentiation genes of these cells, the specificity of their surface markers, or their suppressor mechanisms.

In two recent papers, the nucleotidases CD39 and CD73 have been described as T regulatory cell surface markers, linking the suppressive activity of these cells with previous immunosuppressor models in which adenosine and cAMP had functional relevance for cellular immunoregulation.

KEY WORDS: T regulatory cells/ Treg/ /Nucleotidases/ CD39/ CD37 /Adenosine/ cAMP.

INTRODUCTION

For some years now, we have been witnessing the renaissance of suppressor/regulatory T (Treg) cells. Suppressor T cells were firstly described in 1970 by Richard Gershon[1], who postulated that T lymphocytes were capable of both enhancing and inhibiting immunity (reviewed in 2). However, and despite the enormous experimental effort by many laboratories that ensued for more than ten years, and while the molecular bases of antigen recognition by T lymphocytes could be eventually well established, it was impossible to achieve a clear lineage characterization or clearly determine the molecular mechanism(s) of action of T suppressor cells. Then, suppressor T cells simply dissapeared in a mist of scientific discredit.

More than a decade had to pass until the pioneering reports by Sakaguchi et al.[3] demonstrated that, without the suppressor capacity of "natural", thymus-derived $CD4^+CD25^+$ cells, the transfer of naïve $CD4^+$ lymphocytes induced autoimmune diseases in appropriate recipients. These findings provoked a strong re-emergence of the study of these cells, allowing for the development of methods to measure their activity *in vitro*, and the discovery of surface markers that allowed for the isolation of T lymphocyte populations highly enriched for suppressor cells. It was thus established that they were able of regulating the function of other cells, controlling and/or preventing autoimmunity, infections, and cancer, and raising a great interest towards their therapeutic potential.

It is now clear that, besides those described by Sakaguchi et al., regulatory T cells comprise more than one cell type. In fact, over the years different types of T regulatory cells have been described upon their origin or mode of action: natural, adaptive, Tr1, Th3, etc. Attending to their origin, Treg cells can be classified as natural or adaptive. The former originate in the thymus, and when reaching the periphery they are anergic cells that hardly proliferate in response to antigens.

It has been shown that $CD4^+CD25^-$ T cell populations can also contain cells with potent regulatory activity. These other types of Treg cells have been included under the term "adaptive" or "induced" due their inducible condition in secondary lymphoid organs and even *in vitro;* their mechanism of action appears mediated by the secretion of interleukins like IL-10 (secreted by type 1 Tr, Tr1) or TGF-β (secreted by type 3 T helper cells, or Th3)[4-6] and are implicated in processes of oral tolerance induction to Ag[7, 8].

However, in spite of great investigative effort, and the abundant literature emerging on this issue, we are still beginning to know the nature, the mechanisms and possible means to control these T cells in order to be used with therapeutical purposes.

NATURAL Treg CELLS

Given their natural condition and their thymic origin, these cells are of capital importance to prevent autoimmunity[9]. The therapeutic use of Tregs needs well stablished, efficient means to isolate these cells, as well as methods to expand them without losing their suppressive function. Consequently, finding specific surface markers of Treg cells has been a prime target of study. Characteristically, natural Treg lymphocytes express the alpha chain of the IL-2 receptor CD25, and, *in vitro*, they exert their suppressive action through cell-to-cell contacts.

Treg cells express the forkhead transcription factor FoxP3; this factor is considered a master gene for natural Treg differentiation, and a better marker of this T cell lineage than CD25. Unfortunately, except in the case of certain transgenic mice expressing fluorescent-tagged forms of FoxP3 (see below), its intracellular nature precludes its use as a differentiation marker useful for Treg isolation.

As mentioned above, the therapeutic potential of Treg has driven many laboratories to launch a sustained effort to achieve their isolation and characterization. However, these studies have shown important problems, including the following: i) the absence of exclusive surface markers characteristic for these cells, which would allow an easy isolation by standard methods; ii) the low number of Treg cells in the lymphoid organs or blood of normal individuals; iii) the lack of definitive data concerning growth factors and stimuli controlling the specific expansion of Treg; and iv) their own nature of anergic cells proliferating with difficulties to antigenic stimuli.

THE QUEST FOR Treg MARKERS: CD25 AND FoxP3

Reports from Sakaguchi et al. unveiled the suppressor/regulatory ability of a minor $CD4^+$ T cell population present in the organism, which characteristically expressed the CD25 marker[3, 9]. However, this molecule, which is part of IL-2 receptor, cannot be used as a differentiation marker for these cells due to its expression by other cells like activated T lymphocytes. Thus, many laboratories searched for alternative surface molecules able of identifying Treg cells among the heterogeneous mixture of lymphoid and myeloid cell populations present in lymphoid organs.

The comparison of gene expresssion in $CD25^+$ Treg versus the bulk of $CD4^+$ T cells allowed the identification of the X chromosome-encoded forkhead transcription factor FoxP3 as a key controller of the development and function of natural Tregs. Studies using mice expressing FoxP3 as a fusion protein with green fluorescent protein (GFP) allowed an easy identification of the $FoxP3^+$ cells. It was thus shown that it

serves as a specific lineage marker and confers suppressor activity to Treg cells[10-12]. However, its intracellular location makes it impossible to be used for the isolation of live Treg cells. The differentiation of most Treg cells found in the periphery takes place in the thymus following the induction of FoxP3 in a subset of $\alpha\beta^+$ T cells. Interestingly, these cells scape negative selection although they have an increased affinity for self-peptide-MHC complexes. FoxP3-dependent differentiation of thymic Treg cells need additional signals sent through cytokine receptors containing the common γ-chain[13]. FoxP3 controls regulatory T cell function through cooperation with NFAT[14]. In fact, NF-AT-FoxP3 complexes upregulate the expression of CD25 and CTLA-4.

FoxP3 is expressed in high amounts in peripheral and thymic $CD25^+$ Treg cells in mice; in contrast, FoxP3 expression cannot be detected in $CD25^+$ conventional T cells activated under physiological conditions[10,11,15-17]. However, in humans low levels and transient FoxP3 expression has been observed in some activated conventional T cell population[18]. In the mouse, a low fraction of $FoxP3^+CD25^-$ cells has been detected in BALB/c mice, and this fraction is increased in old mice[19].

As mentioned before, regulatory T cell lineage specification by FoxP3 was demonstrated by Fontenot et al.[11] by using mice harboring GFP-FoxP3 fusion protein-reporter knock-in allele; in this system, $CD4^+CD25^{high}FoxP3^{gfp+}$ and $CD4^+CD25^{low/null}FoxP3^{gfp+}$ populations suppressed $CD4^+$ T cell proliferation with equal potency, while neither the $CD4^+CD25^-Fox^{gfp-}$ cells nor the $CD4^+CD25^+FoxP3^{gfp-}$ cells showed suppressor activity. Thus, FoxP3 expression, rather than CD25 expression, directly correlates with Treg function[11]. However, besides the condition of FoxP3 of being a Treg master gene, its intracellular location limits its use as being a potential marker to facilitate isolation and purification of viable Treg cells.

Treg SURFACE MARKERS: CTLA-4, GITR, AND BEYOND

Treg phenotype has been linked to the expression of other surface markers, like CTLA-4 and glucocorticoid-induced tumour necrosis receptor (GITR). An intriguing feature of $CD25^+CD4^+$ natural Tregs in the thymus and the periphery is that they constitutively express CTLA-4, whereas naïve T cells express this molecule only after activation[9] (reviewed in 20). There is evidence that CTLA-4 expressed by natural Tregs has a key role in Treg-mediated suppression *in vivo* and *in vitro,* and different mechanisms of suppression involving the interaction of CTLA-4 with its ligands CD80/CD86, inducing an indirect suppressive effect through blockade of CD28 co-stimulation, or acting directly on Treg cells, have been proposed[20]. However, the fact that CTLA-4 is expressed in naïve cells after activation makes impossible the use of this surface molecule as a specific marker of Treg cells.

A similar problem arises with GITR, a TNF family molecule which is constitutively expressed in Tregs at a higher level than in other T cells. Functional studies indicate that anti-GITR antibodies can abrogate suppression, and stimulation through GITR might make effector T cells resistant to Treg suppression[21]. However, like CTLA-4 or CD25, GITR is also expressed by $CD4^+$ T lymphocytes upon activation[21-23].

Caramalho et al.[24] have reported that naturally arising $CD25^+CD4^+$ Treg cells in normal mice selectively express several members of the Toll like receptor family (TLR), and that stimulation of these cells through TLRs could expand them and strengthen their suppressive activity.

Depending on the system of study, other surface markers have been defined for Treg cells. These include neuropilin-1[25], CXCR5[26], CD103[27], the chemokine receptors CCR4 and CCR8[28], selectins (CD62L)[29], CD27[30], or CD127[31], among others. However, none of these molecules unequivocally define and identify Treg cells.

The expression pattern of other accessory molecules on $CD25^+CD4^+$ Treg is $CD45RB^{low}$, $CD44^{high}$, $CD5^{high}$, CD54(ICAM-1)^{high}, CD11a/CD18(LFA-1)^{high}, partly $CD62L^{low}$[32]. This pattern suggests that they might be in a "primed", "activated", "effector" or "memory" state, probably due to a constant stimulation by self-peptide/MHCs on APCs in the normal internal milieu. Upon infection, they can be more swiftly recruited to the infection site than other T cells, as recently demonstrated by Belkaid et al.[33].

Recently, a study of gene profiling showed that the ectonucleoside hydrolase enzyme CD39 was one of 67 genes highly associated with FoxP3 expression, even more so than CD25[11]. In a recent publication, Deaglio et al. found that CD39 was expressed differentially by Treg cells together with CD73[34]. Furthermore, its function supports new suppressive mechanisms of Treg cells that will be commented below.

Treg CELLS: MECHANISMS OF SUPPRESSION

Different lines of evidence demonstrate that $FoxP3^+CD25^+CD4^+$ natural Treg cells suppress the activation and/or expansion of multiple types of immunocompetent cells. Initially, Sakaguchi et al. found that Treg depletion induced $CD4^+$ T cell-mediated autoimmune disease, whereas inoculation of Tregs inhibited the development of disease[3]. After that first demonstration, others have shown that Tregs can also act on the function and/or proliferation of $CD8^+$ and B lymphocytes, NK and NKT cells, and DCs (reviewed in 20).

The induction of suppressive activity in Treg cells requires an activation signal through the TCR, but suppression takes

place in an antigen non-specific manner[35, 36]. Whereas one major task of naturally occurring $CD25^+CD4^+$ Treg cells is to inhibit immune responses against self-antigens, it should be kept in mind that they also function to suppress and control immune responses arising against microbes invading or living within the host (reviewed in 32). The critical point then is how do natural $CD25^+CD4^+$ Tregs manage to suppress autoimmune responses or excessive antimicrobial responses causing immunopathology, yet they allow the development of sufficient protective responses against invading pathogenic microbes.

In rodents and humans, suppression by Treg *in vitro* depends on a cell-to-cell contact mechanism, as shown by the finding that Treg suppression is abolished when Tregs and responder cells are separated by a semi-permeable membrane[37,38]. Since then, various molecular and cellular events have been described to explain how Treg can suppress immune responses, some of them including the participation of co-stimulatory molecules expressed by Treg or APC, which can contribute to regulate their expansion and suppressive activity. A role for CTLA-4 seems definite since: a) $CD25^+CD4^+$ Tregs constitutively express CTLA-4 and b) this appears to be required for their activation, as specific blockade of the interaction between CTLA-4 on Treg and CD80/CD86 on APCs abrogates suppression. In contrast, active signalling through CD28, along with TCR stimulation, counteracts and neutralizes Treg suppression[3].

CTLA-4 has about 100-fold affinity for CD80/CD86 molecules than CD28, and based on these findings the following scenario can be envisaged for the roles of Treg in controlling immune responses in the normal state or upon infection (as proposed in 32). In the absence of infection, APCs express basal levels of CD80/CD86 and a low level of class II MHC binding self-peptide. $CD25^+CD4^+$ Treg with self specificity might be activated by recognizing peptides while receiving a signal through CTLA-4, but not through CD28, and thereby suppress autoreactive T cells. Upon microbial infection, APCs are activated and increase their expression of CD80/CD86 as well as microbe-derived peptides bound to class II MHC molecules. $CD25^+CD4^+$ Tregs recognizing the peptides will receive a signal through CD28 in addition to CTLA-4, lessen their suppressive activity, and allow the activation/expansion of effector T cells capable of eliminating microbes. In synergy with CD28-signalling, IL-2 from responding effector T cells and a signal through GITR would also act to attenuate the suppressive activity of Tregs. With the elimination of microbial peptides and decreased CD80/CD86 expression, Tregs would revert to their original state and reacquire the basal regulatory activity[32].

Recently, a direct role of CTLA-4 on Treg function has been described, which implies that CTLA-4 interacting with their CD80/CD86 induces the enzyme indoleamine 2,3-dioxygenase (IDO) in APC like dendritic cells (DCs)[39]. This enzyme catalizes the conversion of tryptophan into metabolites having a potent immunosuppressive effect in the local environment of DCs by means of cytotoxicity, or perhaps by inducing *de novo* generation of Tregs from naïve $CD25^-CD4^+$ T cells.

TLRs also play an important role in Treg activation. $CD25^+CD4^+$ T cells in normal naive mice selectively express TLR 4, 5, 7 and 8 (TLR1, 2, and 6 are more broadly expressed and are not confined to Treg). TLR4 acts as a receptor for bacterial LPS. *In vitro* stimulation of $CD25^+CD4^+$ T cells with LPS elicits their proliferation, prolongs their survival and augments their *in vitro* suppressive activity even in the absence of APCs, indicating that LPS directly acts on TLR4 molecules expressed by Treg cells[24].

Cytokines also play an important role in the control of Treg activation and function. For instance, a high dose of IL-2 along with TCR stimulation triggers their proliferation and neutralizes their suppressive activity during their proliferation[37]. Such expanded Tregs show more potent suppressive activity than before expansion[35]. The main source of IL-2 for Treg survival seems to be other T cells. Linked with this data, another mechanism of Treg-mediated suppression is that Tregs might adsorb IL-2 to their high affinity receptor, thereby blocking proliferation of other T cells. Nevertheless, $FoxP3^+$ T cells from IL-2-receptor-deficient mice suppress *in vitro* as much as IL-2 receptor-intact Tregs[40].

In contrast with the requirement of cell contact for Treg suppression *in vitro*, there are many reports indicating that cytokines such as IL-10 and TGF-β are needed *in vivo* for mediating suppression or conditioning a suppressive milieu (reviewed in 20).

Thus, several mechanisms have been described to explain how Treg can suppress immune responses; however, none of those mechanisms can explain all aspects of suppression. It is likely that different combinations of suppressive mechanisms are operative, depending on the precise milieu and the type of immune response. The existence of a single key mechanism that has not been found yet is also possible.

NUCLEOTIDE METABOLISM DERIVATIVES: A KEY TO UNDERSTAND Treg FUNCTION?

Recently, two different papers by Deaglio et al.[34] and Bopp et al.[41], focus their attention on the nucleotide metabolism as a biochemical key to understand the regulatory function of natural Treg cells. In fact, these papers suggest that the coordinated expression of CD39/CD73 on Treg cells and the adenosine A2A receptor on activated effector T cells set immunosuppressive loops to work.

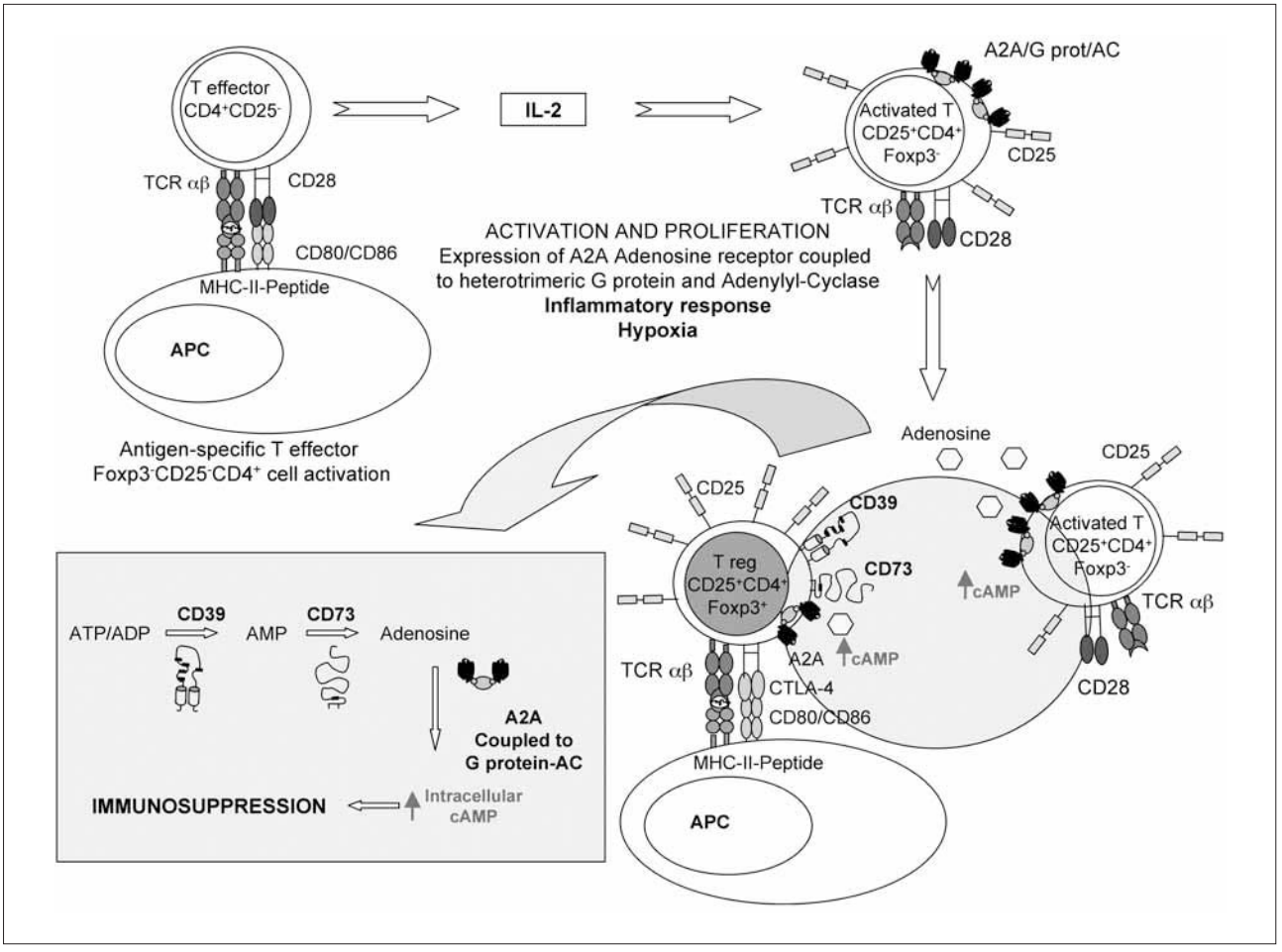

Figure 1. *A panoramic overview of mechanisms of inflammation control involving natural Treg cells, as deduced from the recent reports by Deaglio and Bopp and previous work commented in the text.*
Activation of effector CD25-CD4+ T cells by professional Ag-presenting cells drives them to IL2 secretion and proliferation, leading to an inflammatory response by activated T FoxP3-CD25+CD4+ cells, cellular damage, and hypoxia. Hypoxia induces upregulation of the adenine nucleotide-metabolizing ectoenzyme cascade in which ectoenzymes CD39 and CD73 from natural Treg participate by increasing adenosine accumulation. Adenosine type 1 purinergic G protein-coupled cell surface receptors A2A expressed on Treg act as "sensors" of tissue damage and are upregulated in activated FoxP3- T cell. An OFF signal through A2A receptors is activated in FoxP3- as well as in FoxP3+ natural Treg cells leading to increased cAMP levels. cAMP is then transferred to FoxP3-CD4+ T effector cells through gap junctions. Elevated levels of cAMP inhibit activated immune cells in a negative feedback to prevent additional tissue damage.

Based on gene clustering models, Gavin et al.[12] predict that there are at least three potential Treg cell mechanisms derived from a) the secretion of suppressive soluble factors; b) from the generation of extracellular adenosine; and c) from the release of reactive oxygen. Previous data had shown that several type 1 purinergic (adenosine) receptors including A2A could induce extra- and/or immediate pericellular accumulation of adenosine inducing immunosuppressive cellular responses[42]. Interestingly, genetic data also indicate that adenosine, which is operative via the A2A adenosine receptor, plays a critical role in inhibiting effector functions of activated T cells[43,44]. In fact, these data prompted studies showing that adenosinergic mechanisms could modulate the inflammation in models of T-cell dependent autoimmune and viral hepatitis[45], in antitumour T cell immunity[46], or in experimental models of T cell-mediated colitis[47].

In the recent paper by Deaglio et al.[34], the authors propose that Treg cells are in charge of extracellular nucleotide catabolism regulation in such a way that they might be responsible for the coordination of such adenosinergic effects. In their study, they have determined the generation of pericellular adenosine, which is catalysed by CD39 (ectonucleoside triphosphate diphosphohydrolase-1; EC 3.6.1.5), an ectoenzyme that hydrolyzes ATP/UTP and ADP/UDP to the respective nucleosides such as AMP. Extracellular nucleoside monophosphates are, in turn, rapidly degraded to nucleosides

(i.e. adenosine) by soluble or membrane-bound ecto-5´-nucleotidases (i.e. CD73, [EC 3.1.3.5][48] (a scheme of the proposed mechanisms is depicted in Fig. 1).

CD39 AND CD73

The study by Deaglio et al.[34], reports the identification of CD39 together with CD73 as novel cell surface markers of $CD4^+$ Treg cells in the mouse. CD39 is expressed by the majority of monocytes and by a subset of lymphocytes, most of which are B lymphocytes. The remaining $CD39^+$ cells comprise 8-12% of the $CD4^+$ subset that concurrently expresses CD25. Thus, gene expression profiling of $CD4^+$ cells, sorted on the basis of CD39 cell surface expression, indicates that $CD4^+CD39^+$, but not $CD4^+CD39^-$ T cells, express high levels of FoxP3, CD25, GITR and CTLA-4 transcripts, which is in full agreement with the previously established Treg cell phenotype. Furthermore, and as expected from the previous data, this population inhibits the proliferation of $CD4^+CD25^-$ lymphocytes, as shown by standard *in vitro* suppression assays.

It is also very interesting that, according to Deaglio et al., $CD4^+CD39^+$ and $CD4^+CD25^+$ subsets do not completely overlap, whereas $CD4^+CD39^+$ and $CD4^+FoxP3^+$ do. In this regard, as mentioned above, recent data demonstrate that FoxP3 correlates better with Treg function than CD25, and FoxP3 is expressed in a significant percentage of $CD4^+$ cells that is $CD25^-$[11, 19].

On the other hand, the ecto-5´-nucleotidase CD73, responsible for the degradation of extracellular nucleoside monophosphate to nucleoside, is expressed by $CD25^+FoxP3^+$ Treg cells that, by means of the generation of adenosine, dampen inflammation[49]. Using $FoxP3^+(GFP^+)$ knock-in mice, Deaglio et al.[34] report that Treg cells coexpress the ectonucleosidases CD39 and CD73, which provide precise phenotypic markers and define biochemically relevant mechanisms of Treg cells. It should be mentioned that although a small population of $CD4^+FoxP3^-CD39^+$ cells does exist, it does not express CD73 mRNA and fails to generate adenosine from ADP. $CD8^+$ T cells express CD73 but not CD39 and they also fail to generate adenosine.

The suppressive effect of adenosine is mediated through its binding to adenosine type 1 purinergic G protein-coupled cell surface receptors, which are termed A1, A2A, A2B and A3. The A2A receptor has been identified as the main inflammatory adenosine receptor associated with T cells[45]. In resting conditions, the highest levels of A2A mRNA are found in Treg cells, which, in the presence of adenosine generated by CD39, may account for the anergic state typical of this population. Minimal amounts of A2A adenosine receptor mRNA could be detected in $CD4^+CD25^-$ cells, but effector T cells acquire A2A receptors during activation (after day 4 and peaking at day 6)[34]. The timing of up-regulation of the A2A receptor during T cell activation suggests that this novel immunosuppressive loop is functional during the late phases of T effector cell activation and proliferation, likely coinciding with maximal nucleotide leak from increasing hypoxic or activated cells[50].

The results obtained in *in vitro* systems were analysed *in vivo* by using CD39-null and A2A-null mice. Using these mice, Deaglio et al.[34] demonstrate that $CD4^+$ cells from CD39-null show markedly diminished rates of ADP phosphohydrolysis with no production of adenosine and at the same time show impaired suppressive properties *in vitro* and fail to block allograft rejection *in vivo*. Thus, these studies add relevant data to understand the impact of CD39 in the suppressive functions of Treg cells and may help in developing novel therapeutic approaches targeting inflammatory and autoimmune disorders.

CYCLIC AMP IS A KEY COMPONENT OF REGULATORY T CELL-MEDIATED SUPPRESSION

Another report by Bopp et al.[41] reinforces the importance of nucleotide metabolism in Treg function, demonstrating that naturally occurring Treg harbor high levels of cAMP, which is "per se" a potent inhibitor of proliferation and IL-2 synthesis in T cells. The inhibition of IL-2 gene expression in the responder T cells is a characteristic feature of natural Treg cell-mediated suppression[38]. It is well known that cAMP is a potent inhibitor of T cell growth, differentiation, and proliferation[51], but the mechanisms involved in cAMP-mediated suppressive functions are largely undetermined. Upon coactivation of responder T lymphocytes with naturally occurring Treg cells, the cAMP content of the former is strongly increased. The results obtained by Bopp et al.[41] describe a model in which cAMP is crucial for naturally occurring Treg cell-mediated suppression and transverses membranes via gap junctions. This model may help in explaining the cell-to-cell contact necessary for Treg function in some systems.

This analysis reveals for the first time an unexpected role for a classical second messenger in combination with an ubiquitous system of intercellular communication in naturally occurring Treg cell-mediated suppression. Results by Deaglio and by Bopp[34, 41] may fit together in a classical model of control of tissue damage by adenosine A2A receptors. It is known that the generation of intracellular cAMP is triggered by A2A receptors acting in a delayed negative feedback manner to prevent tissue damage[52]. Tissue damage-associated deep hypoxia induces upregulation of the adenine nucleotide-metabolizing ecto-enzyme cascade, comprising

CD39 and CD73; the resulting accumulation of adenosine triggers an OFF signal through A2A receptor in immune cells. In these regulatory mechanisms, oxygen deprivation and extracellular adenosine accumulation serve as "reporters", while A2A adenosine receptors serve as "sensors" of excessive tissue damage. The A2A receptor-triggered generation of intracellular cAMP then inhibits activated immune cells in a delayed negative feedback manner to prevent additional tissue damage[52]. cAMP expressed at high levels in Treg cells can then be transferred to responder T cells by gap junctions, increasing the regulatory loop of inflammation control. A panoramic overview of these mechanisms of inflammation control is depicted in Fig 1.

The complicated puzzle of Treg control mechanisms is starting to fit. Many new concepts and mechanisms have been brought to light since the concept of suppressor or regulatory T cells was rescued, but it is not easy to find needles in a haystack.

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DISCLOSURES

The authors declare no financial conflict of interest.

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