AIFM1 (Apoptosis-Inducing Factor, Mitochondrion-Associated, 1)

▶ AIF

A-Kinase Anchoring Protein (AKAP)

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AKAPs

AKAPs are a diverse family of scaffold proteins that form multi-protein complexes, integrating 3'-5'-cyclic adenosine monophosphate (cAMP) -signaling with protein kinases, phosphatases, and other effector proteins.

AKAP Historical Background

Early physiology experiments illustrated that stimulation of 3'-5'-cyclic adenosine monophosphate (cAMP) synthesis by different agonists mobilize cAMP-dependent protein kinase; PKA to elicit distinct physiological outputs, even within the same tissue. For example, adrenergic stimulation selectively activates a pool of PKA associated with the particulate fraction of isolated cardiomyocytes, while prostenoids predominantly activate cytosolic PKA. These observations led to the concept of compartmentation of PKA signaling inside cells (reviewed in Steinberg and Brunton 2001). Initial evidence supporting this concept came from experiments demonstrating that type II PKA co-purifies with microtubules as a consequence of protein–protein interactions between regulatory RII subunits of PKA and microtubule-associated protein MAP2 (the first identified AKAP) (Theurkauf and Vallee 1982).

In the order of 40 RII-binding proteins have since been identified (Welch et al. 2010). The majority of these proteins have been identified through the use of the RII overlay technique and a variety of interaction cloning strategies.

The identification and characterization of AKAP family members has allowed the comparison of their primary sequences as well as their subcellular distribution. Almost all AKAPs bind to the RII dimer of PKA through a well-conserved amphipathic α-helical motif (Carr et al. 1991). The majority of known AKAPs bind specifically to the RII holoenzyme, however, several dual specificity AKAPs, which bind to both PKA subtypes, have also been identified. These include the dual-function anchoring proteins D-AKAP1 and 2 (Huang et al. 1997).

Analysis of AKAP subcellular location shows that each AKAP has a unique distribution within a cell type that is generally conferred by a targeting motif (Colledge and Scott 1999).

AKAPs are recognized as diverse proteins that assemble multi-protein complexes, to integrate cAMP-responsive events with other signaling processes. For example, AKAP79 binds PKA and also interacts with protein kinase C (PKC) and protein phosphatase 2B (PP2B/calcinuerin) (Klauck et al. 1996). There are now many examples of AKAPs that coordinate enzymes with opposing actions, such as ▶ adenylyl cyclases and phosphodiesterases.

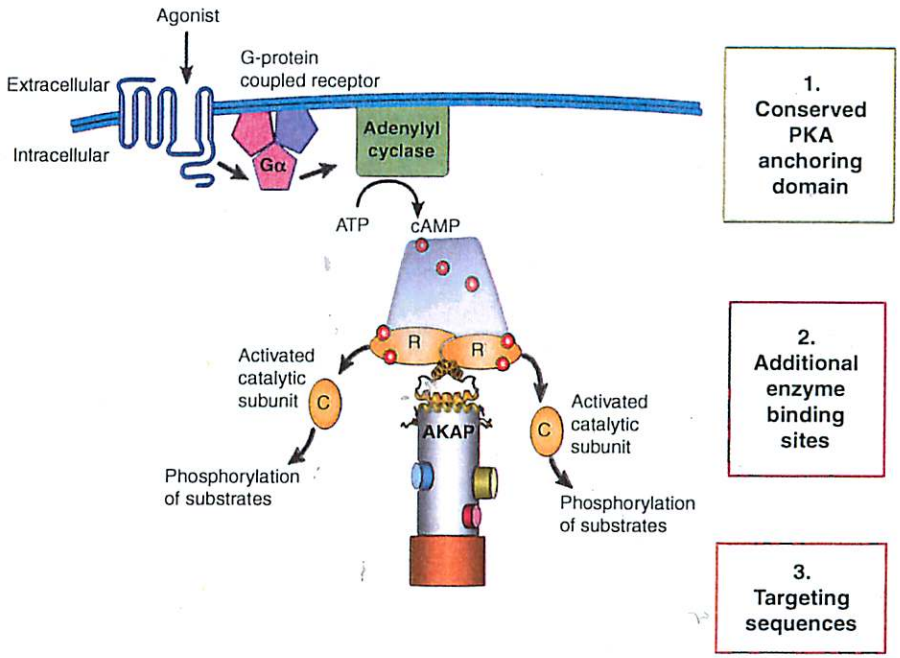
The historical perspective described here is summarized in Fig. 1. See Smith et al. 2006 for additional details.

Properties of AKAPs

As depicted in Fig. 2, all members of the AKAP family possess:

1. A conserved protein kinase A (PKA) anchoring domain.
2. Binding sites for additional signaling components. For example, AKAPs act to directly couple PKA to upstream activators of the cAMP cascade (i.e., β-adrenergic receptors and ▶ adenylyl cyclase), signal terminators (i.e., phosphodiesterases and protein phosphatases), and other elements of signal transduction pathways (i.e., protein kinases, calmodulin, and small molecular weight GTPases).
3. A targeting domain, functioning to compartmentalize signaling complexes to distinct subcellular locations, thereby generating substrate specificity (Colledge and Scott 1999).

A-Kinase Anchoring Protein (AKAP), Fig. 1 *Major discoveries in the study of compartmentalized cAMP signaling and AKAP function.* For further detail, see accompanying text and review by Smith et al. 2006



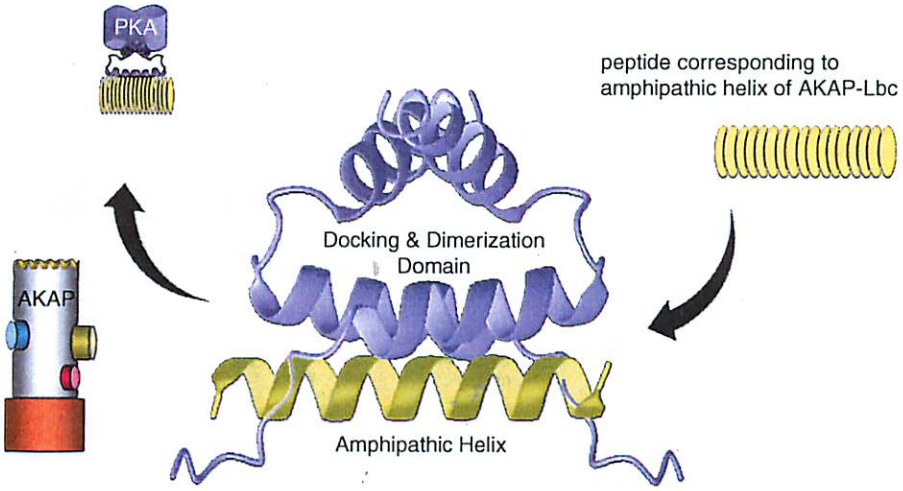
A-Kinase Anchoring Protein (AKAP), Fig. 2 *Properties of AKAPs.* AKAPs regulate the subcellular localization of PKA, thereby generating substrate specificity for PKA. AKAPs have three general properties: (1) AKAPs possess a conserved PKA anchoring domain. (2) AKAPs also bind additional signaling proteins (e.g., other protein kinases, protein phosphatases, phosphodiesterases, adenylyl cyclases, and small G proteins). (3) AKAPs possess targeting sequences directing signaling complexes to discrete subcellular locations

AKAP Nomenclature

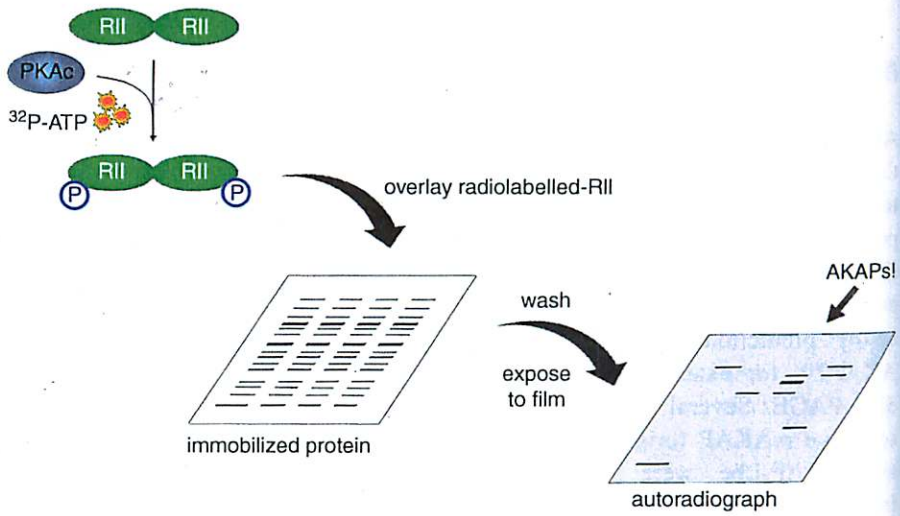
AKAPs have little primary sequence similarity and thus are classified purely on the basis of their ability to bind PKA (Carr et al. 1991). These anchoring proteins were originally named according to their apparent molecular mass determined by SDS polyacrylamide gel electrophoresis (SDS-PAGE) or by prediction from the open reading frame: AKAP79, for example, migrates at ~79 kDa by SDS-PAGE. Several AKAPs, such as the muscle-selective mAKAP (originally known as AKAP100) and AKAP-Lbc, were subsequently found to be fragments or smaller transcripts of larger genes and were renamed. More recently identified AKAPs, for example, Gravin, Ezrin, Rab32, WAVE-1, SKIP and cardiac Troponin T retain their original designations. In the context of nucleotide and protein database nomenclature, AKAPs are numbered sequentially (e.g., AKAP79 is termed "AKAP5"). More recent AKAPs with different names, such as Ezrin, Rab32, WAVE-1, and cTnT have not been included in this classification. See Pidoux and Tasken 2010 and Welch et al. 2010 for comprehensive tables of AKAPs (with gene nomenclature committee names).

Techniques for Identification of AKAPs and Disruption of AKAP-Mediated Protein Kinase a Signaling

Most AKAPs contain a recognizable hallmark sequence (approximately 20 amino acid residues, predicted to form an amphipathic helix) that forms a binding site for the R subunits. Structural studies indicate that the hydrophobic face of this region fits into a binding pocket formed by the N-terminal regions of the RII dimer of PKA (Newlon et al. 1997). Cellular delivery of a peptide or related derivatives (cell-soluble stearated forms, or plasmid-based expression) originally based on the RII-binding region in AKAP-Lbc has become a standard means to establish whether anchored pools of PKA participate in various cAMP signaling events by disrupting PKA-RII anchoring inside cells (see Fig. 3). The utility of this peptide as a disruptor of PKA anchoring was first demonstrated in studies showing that perfusion of this peptide into cultured hippocampal neurons disrupts the localized phosphorylation of the α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA)-type glutamate receptor by anchored PKA. The functional consequence of this disruption was to decrease the responsiveness of the ion channel to synaptic signals (Rosenmund et al. 1994).



A-Kinase Anchoring Protein (AKAP), Fig. 3 *Study of AKAP function by peptide-mediated disruption of protein kinase A anchoring.* The AKAP-amphipathic helix-PKA-RII interaction can be disrupted in vivo by introduction of a competing peptide (originally called Ht31). The disruptor peptide will bind to PKA-RII, thereby displacing PKA from an AKAP inside a cell. Mislocalization of PKA by displacement from an AKAP may lead to uncoupling of site-specific PKA signaling. This has been demonstrated in the processes of channel and receptor regulation, insulin secretion, sperm motility, and oocyte maturation



A-Kinase Anchoring Protein (AKAP), Fig. 4 *Identification of AKAPs by RII overlay.* Purified PKA regulatory subunit RII is radiolabeled with γ-[^{32}P]-ATP and overlayed on to a membrane with immobilized protein. Following incubation with RII and extensive washing of the membrane, AKAPs are identified by autoradiography

The high affinity interaction between AKAPs and the RII subunit dimer of PKA also underlies the RII overlay procedure (shown in Fig. 4), which has been used extensively, with great success, to identify AKAPs.

AKAP Function

AKAPs have been implicated in diverse physiological processes (reviewed in Carnegie et al. 2009), including reproduction and development, learning and memory, cardiac function, and diseases such as cancer and diabetes.

AKAPs, and Reproduction and Development

AKAPs function in the regulation of motility, sperm capacitation, the acrosome reaction, and oocyte maturation. As oocytes undergo meiosis, a change in PKA localization is observed (Rawe et al. 2004), through the expression and localization of different AKAPs. Thus PKA activity is specifically targeted to specific sites and substrates in the oocyte.

Gravin (*AKAP12*) has been implicated in embryogenesis, regulating cell migration through inhibition of a Rho/ROCK/myosin II pathway (Weiser et al. 2007).

AKAPs, and Learning and Memory

One of the first physiological roles identified for AKAPs was the synchronization of synaptic signaling events that underlie learning and memory. AKAP79 (*AKAP5*) (or the mouse ortholog, AKAP150) can regulate synaptic plasticity by coordinating PKA, PKC, and PP2B/calcineurin at the post-synaptic membrane (Klauck et al. 1996). Phosphorylation of channel subunits modulates synaptic efficiency either by regulating the conductance of ion channels or by regulating surface expression of the channel complex.

WAVE-1 (Wiskott–Aldrich syndrome, verprolin-homology domain containing protein) is another AKAP with defined neuronal functions; WAVE-1 null mice display defects in hippocampal learning and memory (Soderling et al. 2003). Expression of the WAVE-1 isoform is restricted to the central nervous system where it functions to organize protein networks involved in the regulation of the actin assembly and synaptic plasticity. WAVE-1 is likely to exist in many different protein complexes, relating to its spatiotemporal function. For example, the RII-binding region of this protein overlaps with the actin-binding domain, whereas the C-terminal region of the protein interfaces with the Arp 2/3 complex, a constellation of actin-related proteins that control changes in cytoskeletal shape.

Cardiac AKAPs

As depicted in Fig. 5, several AKAPs have been identified in the heart. These "cardiac" AKAPs have been implicated in the regulation of cytoskeletal proteins and cardiac ion channels, that coordinate excitation-contraction (EC) coupling. Thus AKAPs mediate cardiac inotropy, chronotropy, and lusitropy. For example, the long splice variant of *AKAP7*; AKAP15/18δ targets PKA to phospholamban, which is a critical regulator of the sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA) (Lygren et al. 2007), leading to the effects of adrenergic stimulation on calcium reuptake.

AKAP79 (*AKAP5*) also plays a role in the regulation of Ca^{2+}, specifically by targeting PKCα to the L-type Ca^{2+} channel in arterial myocytes. Recent studies demonstrate that AKAP150 null mice were found to lack persistent Ca^{2+} sparklets and have lower arterial wall intracellular calcium and decreased myogenic tone. These null mice were hypotensive and did not develop angiotensin II–induced hypertension (Navedo et al. 2008).

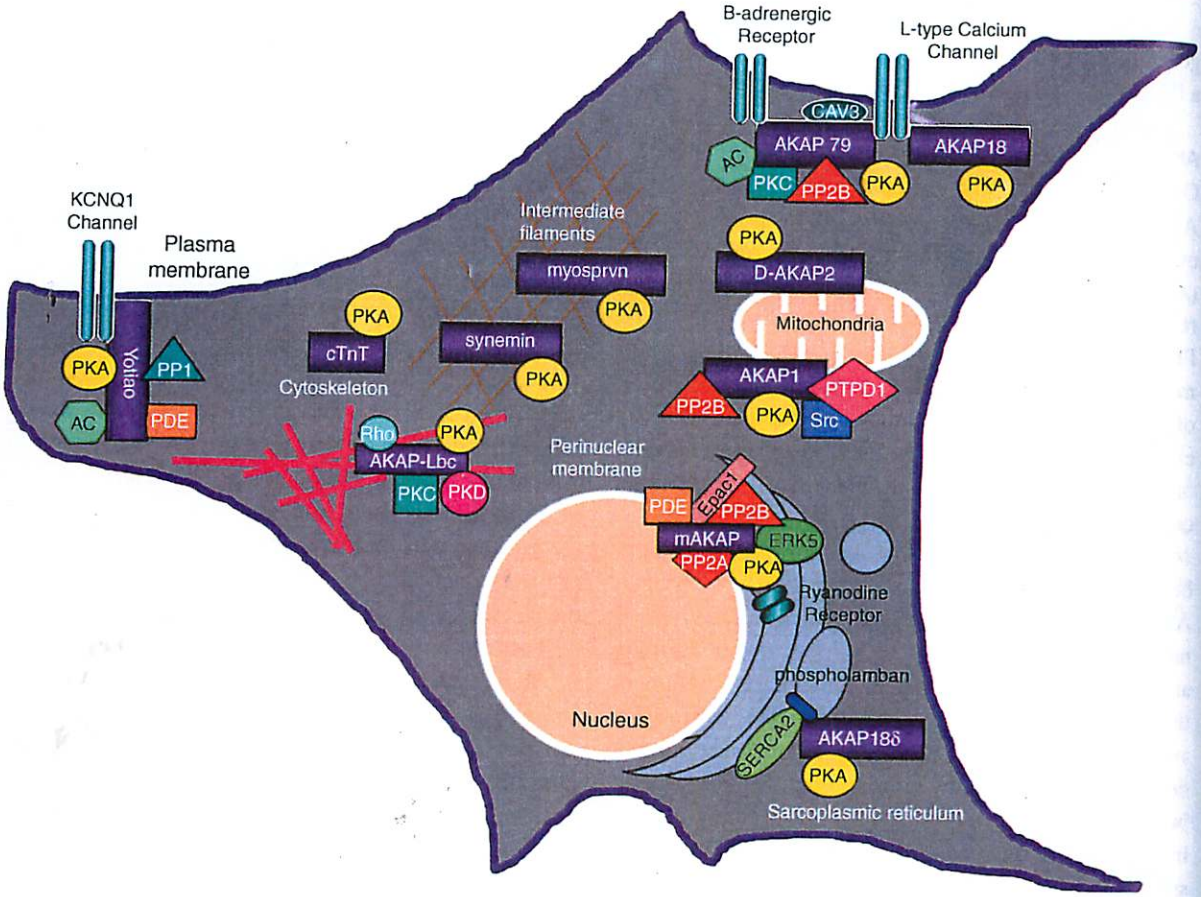
Interestingly, a mutation in Yotiao (*AKAP9*) has been identified in patients with familial long-QT syndrome (LQTS) (Chen et al. 2007). Long QT syndrome (LQTS) is a congenital disorder characterized by a prolongation of the QT interval on ECG and often, ventricular tachyarrhythmias, which may lead to cardiac arrest. Yotiao forms a macromolecular complex (targeting PKA, PP1, and PDE4D3) with the slowly activating cardiac potassium channel I_{Ks}, which is critical for repolarization of the ventricular action potential in the heart. Mutations in either yotiao or the I_{Ks} channel subunits that disrupts their interaction cause a reduction in PKA-mediated phosphorylation of the channel, leading to prolonged ventricular action.

The muscle-specific A kinase–anchoring protein (mAKAP; *AKAP6*) is highly expressed in cardiac tissue and localized to the perinuclear membrane and junctional sarcoplasmic reticulum where it is in close proximity to a variety of substrates such as L-type Ca^{2+} channels and the ryanodine receptor (RyR), thereby functioning in the regulation of contractility. mAKAP scaffolds multiple signaling molecules including PKA, PDE4D3, Epac1, ERK5, PP2A, and PP2B (calcinuerin). mAKAP has also been implicated in cardiac hypertrophy through ERK5 and calcineurin signaling (Bauman et al. 2007).

AKAP-Lbc (*AKAP13*) also plays a role in the induction of hypertrophy, through integration of multiple signal transduction components including Rho and ▶ PKD (Appert-Collin et al. 2007; Carnegie et al. 2008).

AKAPs and the Immune System

cAMP-PKA signaling is well established as a potent negative regulator of T-cell immune function. Prostaglandin E_2 (PGE2) and other ligands promote the production of cAMP, which in turn activates PKA to inhibit TCR-induced T-cell proliferation. Type I PKA is the predominant PKA isoform in T cells and plays a prominent role in immunomodulation. Studies examining the role of RIIα in the immune system in vivo show that T-cell development, homeostasis, and the generation of a cell-mediated immune response are not altered in RIIα null mice. Recently, the dual specificity



A-Kinase Anchoring Protein (AKAP), Fig. 5 *Cardiac AKAPs*. AKAPs are important in the regulation of cardiac function. AKAP18, AKAP79, and yotiao function in channel regulation, while myospryn, synemin, and cTnT act to target PKA to actin filaments, regulating contractility. mAKAP, AKAP121, and AKAP-Lbc play a role in pathological cardiac hypertrophy

AKAP ezrin has been identified, acting to target type I PKA to the TCR-CD3 complex present at membrane microdomains (lipid rafts) in T cells. Targeting of PKA by ezrin facilitates the phosphorylation and activation of the tyrosine kinase Csk. In turn, ▶ Csk negatively regulates Lck tyrosine kinase activity and T-cell receptor activation (Mosenden and Tasken 2011).

AKAPs and Disease

Several SNPs have been identified in patients with different diseases. For example, mutations identified in the gene encoding the AKAP pericentrin (*PCNT*) were demonstrated to cause Seckel syndrome (Rauch et al. 2008). Seckel syndrome is a disorder associated with defective ATR-dependent DNA damage signaling, resulting in a marked reduction of brain and body size. While the mechanism underlying this disorder is not fully understood, the authors demonstrated that collectively, these mutations result in the loss of expression of all mammalian *PCTN* isoforms.

Cancer

Gravin (*AKAP12*) is down-regulated in a number of tumor types including prostate, ovarian, and breast cancer and is associated with metastatic progression of these tumors, providing evidence supporting the role of gravin as a tumor suppressor (Gelman 2002). The observed role of gravin in embryogenesis also supports a tumor supressor function for this AKAP. It is thought that gravin may act to inhibit the migratory movements observed in some tumors that may cause cell invasion and metastasis.

A truncated form of AKAP-Lbc (*AKAP13*) missing both N- and C-terminal regulatory sequences was originally identified as an oncogene from myeloid leukemia patients.

Diabetes

A role for AKAPs in the regulation of hormone (GLP-1)-mediated insulin secretion was first identified in studies using the PKA-AKAP disruptor peptide (Lester et al. 1997). Results demonstrate that insulin secretion can be regulated by the reversible phosphorylation of β-cell proteins through the AKAP79 targeted effects of PKA and PP2B. More recently, AKAP18α or γ has also been implicated in the regulation of glucose-stimulated insulin secretion (Josefsen et al. 2010).

Summary

AKAP-mediated kinase anchoring is acknowledged as a vital means to synchronize spatial and temporal aspects of signal transduction. In addition, AKAPs are now regarded as signaling nodes that integrate a variety of intracellular signals to modulate a plethora of cellular processes. With the extensive molecular, biochemical, and cellular characterization of many AKAPs, in combination with large functional genomic screens and the study of tissue or whole animal models, the future of this field likely lies in precisely defining how specific AKAPs play their part in maintaining normal physiology and what happens to AKAP signaling complexes in disease states.

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Alpha E Integrin

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Synonyms

Alpha polypeptide; CD103; Human mucosal lymphocyte antigen 1

Historical Background

CD103 (integrin αEβ7) was first identified through the binding of a monoclonal antibody (HML-1, human mucosal lymphocyte antigne-1) to a population of lymphocytes that is preferentially associated with gut epithelium (Cerf-Bensussan et al. 1987). It was later identified that HML-1 bound to CD103 which was expressed predominantly on CD3+ CD8+ T cells, and the vast majority of these cells were found in the intestinal mucosa (Russell et al. 1994). Several functionally distinct epitopes were identified. The HML-1 and αE7-1 epitopes were found to function as costimulatory molecules in lymphocyte proliferative responses to a breast cancer epithelial cell line while the αE7-2 and αE7-3 epitopes did not provide such costimulation (Russell et al. 1994).

It is now clear that CD103 is a classic integrin heterodimer composed of the β7 and αE integrin (CD103) subunits. As described above, early studies identified CD103 as an adhesion molecule expressed exclusively by CD8+ T cells in the gut mucosa; however, subsequent studies revealed that CD103 is also expressed by peripheral CD8+ T cells and diverse leukocyte subsets. Recent studies indicate that CD103 is promiscuously expressed by different leukocyte subsets with known immune functional capabilities including not only CD8+ T cells but also interstitial dendritic cells and regulatory T cells. This review is focused on the mechanisms by which CD103 expression is regulated by these leukocye subsets, and the functional impact of CD103 expression on CD8+ T cells, dendritic cells, and regulatory T cells (Tregs). The therapeutic potential of CD103 blockade is also discussed.

Regulation of CD103 Expression

The precise mechanisms regulating CD103 expression by the different leukocyte subsets remain poorly defined. A leading hypothesis is that leukocytes expressing the CD49d/β7 integrin (i.e., gut homing CD8+ T cells) downregulate CD49d and upregulate CD103 in the presence of bioactive transfoming growth factor beta (TGF-β) to generate CD103 expressing cells. There is also evidence that TGF-β directly induces transcription of the αE gene (Itgae) (Robinson et al. 2001). Regardless of the mechanisms involved, it has been clear that bioactive TGF-β plays a dominant role in regulating CD103 expression since the initial reports on the subject by Kilshaw and Murant nearly 20 years ago (Kilshaw and Murant 1990). A key role for TGF-β in regulating CD103 expression by non-CD8 cells is supported by the observation that conversion of naive T cells into CD4+ CD25+ T regs is dependent on TGF-β activity (Coombes et al. 2007). Similarly, TGF-β induces CD103 expression on CD8 T effectors elicited to allogeneic spleen cells cocultured with TGF-β, and CD103 expression by CD8+ T effectors elicited to allogeneic epithelial cells is blocked by TGF-β neutralizing antibody (Hadley et al. 1997). That TGF-β plays a similar role in vivo is supported by the studies of El-Asady et al. who showed that alloreactive CD8+ T cells