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REVIEW

Modulation of T cell immune functions by the prostaglandin E_2 – cAMP pathway in chronic inflammatory states

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Cyclic AMP is the intracellular second messenger for a variety of immunoregulatory inflammatory mediators such as prostaglandin E2, adenosine and histamine that signal to effector T cells from monocytes, macrophages and regulatory T cells. Protein kinase A (PKA) type I localizes to lipid rafts in effector T cells during T cell activation and directly modulates proximal signal events including phosphorylation of C-terminal Src kinase (Csk), which initiates a negative signal pathway that fine-tunes the T cell activation process. The PKA-Csk immunoregulatory pathway is scaffolded by the A kinase anchoring protein ezrin, the Csk binding protein phosphoprotein associated with glycosphingolipid-enriched membrane microdomains and the linker protein ezrin/radixin/moesin binding protein of 50 kDa. This pathway is hyperactivated in chronic infections with an inflammatory component such as HIV, other immunodeficiencies and around solid tumours as a consequence of local inflammation leading to inhibition of anti-tumour immunity.

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Abbreviations
AKAP, A kinase anchoring protein; Cbp, Csk binding protein; Csk, C-terminal Src kinase; EBP50, ezrin/radixin/moesin (ERM) binding protein of 50 kDa; PAG, phosphoprotein associated with glycosphingolipid-enriched membrane microdomains; PKA, protein kinase A; PLCγ1, phospholipase Cγ1; TCR, T cell receptor

Introduction

The common and versatile second messenger cAMP controls numerous cellular processes and is known to be a potent inhibitor of T cell functions such as antigen-induced proliferation and cytokine production (Skalhegg *et al.*, 1992; Aandahl *et al.*, 2002). The cAMP-protein kinase A (PKA) signalling pathway in T cells is initiated by binding of prostaglandin E_2 (PGE_2) and other extracellular ligands such as catecholamines, serotonin, adenosine and histamine to GPCR, leading to activation of AC and the subsequent generation of cAMP from ATP (Kammer, 1988; Hanoune and Defer, 2001; Loza and Penn, 2010; Mosenden and Tasken, 2011). Endogenous cAMP levels in effector T cells are also in part controlled by regulatory T cells as detailed later.

Human regulatory T cells comprise 5–10% of the peripheral CD4+ T cell population (Ng *et al.*, 2001), are functionally characterized by their ability to suppress effector T cells, have an essential role in maintaining immunological tolerance (Sakaguchi *et al.*, 2006) and are implicated in various clinical conditions such as autoimmune disease, cancer and chronic viral infections. Regulatory T cells can be subdivided into naturally occurring regulatory T cells that are derived from the thymus and adaptive or peripherally induced regulatory T cells that develop from naïve T cells in the periphery during immune activation (Bluestone and Abbas, 2003). The role of cAMP in induction of regulatory T cells and mediation of regulatory T cell suppression is getting increasing attention [reviewed in Yaqub and Tasken (2008)]. Naturally occurring regulatory T cells have been shown to have high cAMP levels

which least partly can be explained by the forkhead box P3 (FOXP3)-dependent down-regulation of PDE3B (Gavin *et al.*, 2007) and/or up-regulation of AC 9 by down-regulation of miR-142-3p (Huang *et al.*, 2009), and are shown to mediate their suppressive function by transferring cAMP to effector T cells through gap junctions (Bopp *et al.*, 2007). Furthermore, the ectoenzymes CD39 and CD73 expressed on naturally occurring regulatory T cells may metabolize ATP from dying cells to generate adenosine which acts via the adenosine A2a receptor on activated effector T cells, thereby suppressing their function by intracellular production of cAMP (Deaglio *et al.*, 2007). In addition, continuous antigen exposure to T cells leads to generation of adaptive regulatory T cells (Aandahl *et al.*, 2004). Adaptive regulatory T cells express COX-2, leading to secretion of PGE_2. PGE_2 stimulates FOXP3 expression in regulatory T cells in addition to inhibiting effector T cell function through activation of the cAMP-PKA-C-terminal Src kinase (Csk) signalling pathway (Figure 1B) (Mahic *et al.*, 2006).

Although other effectors of cAMP such as the guanine exchange factor Epac and cyclic nucleotide-gated ion channels have been identified and extensively characterized in other cell types (Kaupp and Seifert, 2002; Gloerich and Bos, 2010), there is little evidence for expression in T cells, although two reports find Epac in Jurkat or primary T cells (Fuld *et al.*, 2005; Gerlo *et al.*, 2006). Consequently, the functionally important effector system in effector T cells appears to be PKA. Ligand-induced changes in cAMP concentration vary in duration, amplitude and localization in the cell. Spatiotemporal organization of cAMP microdomains is accomplished through coupled control of cAMP production by ACs and degradation by cAMP PDEs, a subset of the superfamily of more than 10 classes of cyclic nucleotide PDEs that degrade cAMP and cGMP (Baillie *et al.*, 2005; Willoughby and Cooper, 2007). Targeting of PKA by A kinase anchoring proteins (AKAPs) and integration of a wide repertoire of proteins involved in signal transduction into complex signal networks further increase the specificity required for the precise regulation of numerous cellular and physiologic processes. The cAMP-PKA pathway is strongly involved in the regulation and modulation of immune responses and is the most potent and acute inhibitor of activation of lymphocytes.

Cyclic AMP and PKA regulate immune function at multiple levels. Possible targets for PKA phosphorylation that can modulate an immune response are the transcription factors cAMP response element binding protein, nuclear factor of activated T cells and NF-κB, and upstream targets like HePTP, Ras, Raf, MAP/ERK kinase (MEK), MAPK, phospholipase Cγ1/2 (PLCγ1/2), PLCβ, Csk and RhoA [reviewed in Skalhegg *et al.* (1994); Torgersen *et al.* (2008); Mosenden and Tasken (2011)]. However, based on studies with selective agonists, activation of PKA type I ($RI\alpha_2C_2$; RI, type I regulatory subunit) has been shown to be necessary and sufficient for mediating inhibition of T cell receptor (TCR)-induced T cell activation by cAMP (Skalhegg *et al.*, 1994). Similarly, PKA type I negatively regulates activation of B cells through the B cell antigen receptor (Levy *et al.*, 1996) and NK cell cytotoxicity elicited through specific NK cell receptors (Torgersen *et al.*, 1997). Although PKA can modulate TCR signalling at multiple levels [reviewed in Torgersen *et al.* (2002); Mosenden and Tasken (2011)], the observed inhibitory effects of cAMP on TCR-induced ζ chain phosphorylation point to an important role for Csk, which is the most upstream PKA target reported so far. PKA phosphorylates S364 in Csk, and thereby induces a two- to fourfold increase in phosphotransferase activity of Csk in lipid rafts of T cells (Vang *et al.*, 2001).

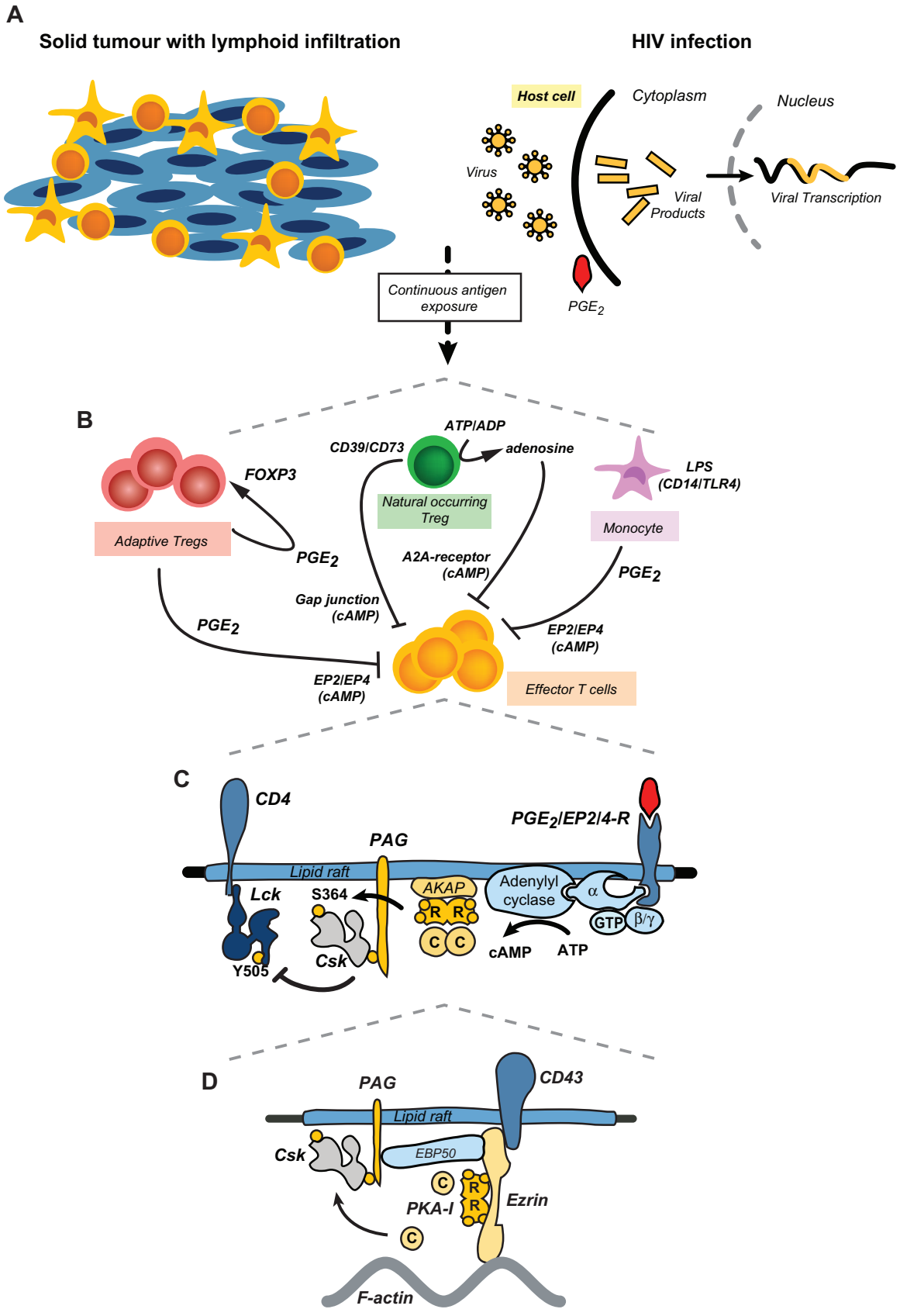
So far, two different mechanisms are reported to regulate Csk activity. PKA, through phosphorylation of S364, increases Csk kinase activity two- to fourfold leading to reduced Lck activity and ζ chain phosphorylation (Torgersen *et al.*, 2001; Vang *et al.*, 2001; Vang *et al.*, 2003). In addition, Csk binding protein (Cbp)/phosphoprotein associated with glycosphingolipid-enriched membrane microdomain (PAG) recruits Csk to the site of action in lipid rafts (Brdicka *et al.*, 2000; Kawabuchi *et al.*, 2000), and the interaction between Csk-Src homology 2 and Cbp/PAG through phosphorylated Y314 increases Csk activity (Takeuchi *et al.*, 2000). Thus, PKA phosphorylation of Csk and Csk interaction with Cbp/PAG may act together in turning on Csk activity, providing a powerful mechanism for terminating activation through antigen receptors dependent on Src kinase signalling.

PKA is targeted to the TCR signalling machinery by interaction with the AKAP ezrin

Ezrin is a 78-kDa protein belonging to the ezrin/radixin/moesin (ERM) family of proteins that plays structural and

Figure 1

Cyclic AMP-mediated induction of Treg cells and suppression of effector T cells. (A) The lymphoid infiltrate around solid tumours is inhibited by peripherally induced regulatory T cells exposed to a chronic antigenic stimulation and inflammation. Similarly, chronic infections such as by the HIV virus provide a continued antigenic stimulation leading to induction of a chronic inflammatory state and immunosuppression. (B) Continuous activation of T cells results in generation of adaptive Tregs that express COX-2, leading to PGE_2 production. Increased PGE_2 levels stimulate further FOXP3 expression in Treg cells in addition to inhibiting effector T cell function. Furthermore, naturally occurring Tregs have high cAMP levels and mediate their suppressive function by transferring cAMP to responder T cells through gap junctions. In addition, the ectoenzymes CD39 and CD73 expressed on naturally occurring Tregs degrade ATP/ADP and catalyze the generation of adenosine, acting via the adenosine A2a receptor on activated effector T cells and thereby suppressing their function by intracellular production of cAMP. Finally, T cell inhibition and induction of FOXP3 due to PGE_2 secretion can also be a result of LPS-activated monocytes secreting PGE_2. (C) In T cells, cAMP inhibits TCR-induced T cell activation and thereby exerts important immunoregulatory functions through a receptor – G protein – AC – cAMP – PKA type I – Csk inhibitory pathway assembled in T cell lipid rafts and acting on the Src family kinase Lck. (D) Ezrin serves to link transmembrane receptors such as CD43 to the actin cytoskeleton via its N-terminal FERM domain and with F-actin via its C-terminus. In T cell lipid rafts, ezrin functions as an AKAP, bringing PKA type I in the proximity to its downstream substrate Csk by forming a supramolecular signalling complex consisting of PKA type I, ezrin, EBP50, Cbp/PAG and Csk.


A
Solid tumour with lymphoid infiltration
HIV infection
Host cell
Cytoplasm
Nucleus
Virus
Viral Products
Viral Transcription
PGE2
Continuous antigen exposure
B
ATP/ADP
CD39/CD73
adenosine
FOXP3
Natural occurring Treg
LPS (CD14/TLR4)
Monocyte
Adaptive Tregs
PGE2
A2A-receptor (cAMP)
PGE2
Gap junction (cAMP)
PGE2
EP2/EP4 (cAMP)
EP2/EP4 (cAMP)
Effector T cells
C
CD4
PGE2/EP2/4-R
PAG
Lipid raft
Lck
S364
AKAP
R R
C C
Adenylyl cyclase
α
GTP
β/γ
cAMP
ATP
Y505
Csk
D
PAG
CD43
Lipid raft
Csk
EBP50
C
R
R
PKA-I
Ezrin
C
F-actin

regulatory roles in the assembly and stabilization of specialized plasma membrane domains by linking microfilaments to the membrane. ERM proteins have a highly homologous N-terminal band four-point-one ERM (FERM) domain that binds directly to a number of transmembrane proteins including CD44 and CD43 (Tsukita *et al.*, 1994; Yonemura *et al.*, 1998) in addition to indirect binding to other membrane proteins via scaffold proteins like ERM binding protein of 50 kDa (EBP50). The actin cytoskeleton interaction is via the C-terminus (Algrain *et al.*, 1993). Most of ezrin's interactions are dependent on conformational activation of the molecule. In the dormant state, binding sites for interaction partners are masked due to an intramolecular interaction between the FERM domain and the C-terminus. Upon phosphorylation of a C-terminal threonine by protein kinase C or Rho kinase, the intramolecular bond is released and other interactions can occur (Bretscher *et al.*, 2002). Mapping studies of ezrin reveal that the PKA RIα binding sequence is located in the α-helical region between the FERM domain and the C-terminus (Ruppelt *et al.*, 2007). Interestingly, ezrin, EBP50 and PAG come together in a complex to scaffold the PKA-Csk inhibitory pathway in T cells (Bretscher *et al.*, 2002).

Functional evidence that PKA type I regulation of T cell responses is dependent on AKAP anchoring by ezrin comes from the observations that disruption of PKA type I binding to ezrin using and anchoring disruptor peptides Ht31 and RI anchoring disruptor (RIAD) or the peptides from the RI specifier region in ezrin displaces PKA type I from lipid rafts and releases the T cells from cAMP-mediated inhibition of proliferation and IL-2 production (Carlson *et al.*, 2006; Ruppelt *et al.*, 2007; Jarnaess *et al.*, 2008). Furthermore, small interfering RNA (siRNA)-mediated knockdown of ezrin abrogated cAMP regulation of IL-2 secretion (Ruppelt *et al.*, 2007), whereas reconstitution with an siRNA-resistant wild-type ezrin, but not an ezrin variant where the PKA binding domain had been mutated, re-established the cAMP-regulation of IL-2 secretion (Ruppelt *et al.*, 2007; Jarnaess *et al.*, 2008). In addition, disruption of the ezrin-EBP50-PAG scaffold at the level of the ezrin-EBP50 interaction also disrupts cAMP-regulation of IL-2 secretion (Figure 1D) (Stokka *et al.*, 2010).

Type I PKA is displaced from AKAPs by the soluble, high-affinity binder RIAD made by combined bioinformatic design and peptide array substitutions starting with a consensus PKA type I binding sequence derived from dual-specificity AKAPs (Carlson *et al.*, 2006). Recently, we disrupted type I PKA anchoring in peripheral T cells by expressing a soluble ezrin fragment with the RIAD sequence inserted in place of the endogenous A-kinase binding domain under the *lck* distal promoter in mice (Mosenden *et al.*, 2011). The RIAD-transgenic mice showed reduced sensitivity to PGE_2- and cAMP-mediated inhibition of effector T cell function, enhanced T cell responsiveness assessed as IL-2 secretion and resistance to murine AIDS, a disease model induced by infection with a mixture of attenuated murine leukaemia viruses (LP-BM5) (Mosenden *et al.*, 2011). Hyperactivation of the cAMP-type I PKA pathway is involved in the T cell dysfunction of HIV infection, and our findings underscores the cAMP-type I PKA pathway in T cells as a putative target for therapeutic intervention in immunodeficiency diseases. Furthermore, our unpublished data suggest that effector T cells protected from Treg suppression through cAMP also show significantly improved anti-tumour immune responses to solid tumours compared to mice with normal type I PKA anchoring (Cornez I, Taskén K, unpubl. data).

Immunological hyperactivation induces a chronic inflammatory state with cAMP-mediated hyporesponsiveness of CD8+ and CD4+ T lymphocytes in HIV-infected patients

HIV causes a chronic infection leading to severe dysfunction of the immune system (Figure 1A) with a markedly increased incidence of opportunistic infections and certain forms of malignancies, ultimately leading to death (Douek *et al.*, 2003). Immune hyperactivation by chronic antigen infusion of non-viable microbial antigens itself leads to functional immunodeficiency in animal models (Hazenberg *et al.*, 2003). Correspondingly, the extent of CD38 expression, as an indicator for hyperimmune activation, has long been recognized as a far better prognostic parameter than HIV RNA levels or CD4 counts (Giorgi *et al.*, 2002; Rodriguez *et al.*, 2006), and assessment of CD38 expression in T cell subsets may further improve prognostic value of CD38 as a surrogate parameter for future CD4 loss and disease progression (Holm *et al.*, 2008). Furthermore, in parallel with CD4 loss and immunodeficiency, the ability to react with new antigens and to boost memory responses becomes seriously impaired. However, signs of hyporesponsiveness can be detected long before clinical immunodeficiency. Moreover, this appears to be true both for HIV-specific cellular immunity and for non-HIV antigens. As to HIV-specific responses, it is known that a large number of HIV-specific CD4+ T cell clones get preferentially infected and rapidly lost in primary infection (Douek *et al.*, 2003). We and others have characterized suppressive HIV-specific regulatory T cells, and further characterized the clinical values of reversing the hyporesponsiveness due to overexpression of programmed cell death-1 on effector T cells, particularly HIV-specific clones (Aandahl *et al.*, 2004; Holm *et al.*, 2008; Torheim *et al.*, 2009; Pettersen *et al.*, 2010).

Impaired responses to common T cell-dependent vaccines such as tetanus toxoid in HIV-infected patients have been reported previously (Kvale *et al.*, 2002). Low vaccine efficacy may be linked to hyperimmune activation, a notion reviewed by Dalgleish (Cadogan and Dalgleish, 2008), and where one important mechanism contributing to poor vaccine responses is dysregulated intracellular signalling in T cells. Elevated intracellular cAMP can indirectly reduce or block T cell activation that is expected after interaction between major histocompatibility complex, TCR and specific antigenic peptide (Mahic *et al.*, 2006). HIV patients have higher levels of PKA-mediated inhibition of TCR signalling and T cell activation than uninfected, healthy controls (Aandahl *et al.*, 1998; 1999). We hypothesized that inhibition of PKA, through decreased production of PGE_2 by COX inhibitors, could reverse this process and restore T cell responsiveness to vaccine antigens. When treating HIV-infected patients with COX-2 inhibitors, we could indeed find increased prolifera-

tive responses (Johansson *et al.*, 2004) and decreased levels of CD38 (Kvale *et al.*, 2006) in patients on highly active antiretroviral therapy and even more pronounced reduction in CD38 as well as tetanus vaccine boosting effects in treatment-naive patients (Pettersen *et al.*, 2011).

Another important source of PGE_2 is monocytes expressing COX-2 (Treves *et al.*, 1982). The latter fits very well with the current concept of increased microbial translocation in chronic HIV which results in high levels of circulating lipopolysaccharides (LPS) (Brenchley *et al.*, 2006), potent inducers of COX-2 enzyme in monocytes. The LPS levels also relate to more rapid disease progression in individual patients.

PGE_2-/COX-2-mediated modulation of anti-tumour immune responses in colorectal cancer

COX-2 levels are elevated in as many as 85% of human colorectal cancers (CRCs) and approximately 50% of colorectal adenomas leading to a chronic inflammation around the cancer (Eberhart *et al.*, 1994). Studies have shown that COX inhibition by non-steroidal anti-inflammatory drugs or aspirin reduces the risk of CRC and may be beneficial in large population groups at risk (Kraus and Arber, 2011). Selective COX-2 inhibitors are also associated with a decline in the incidence of CRC and reduced mortality rate, although COX-2 inhibitors have been associated with serious cardiovascular events in this context (Psaty and Potter, 2006). PGE_2 has been shown to be an important mediator of COX-2 associated effects, and PGE_2 levels are elevated in CRC biopsies compared with normal mucosa and even in patient blood samples (Yaqub *et al.*, 2008). Homozygous deletion of the gene for the PGE_2 receptor prostaglandin E receptor 2 (EP2) reduced the number and size of colorectal polyps in a polyposis mouse model (Fujino *et al.*, 2011). Beside an anti-angiogenic effect (Fujino *et al.*, 2011), COX inhibition promotes apoptosis and alters tumour growth (Sheng *et al.*, 2001) and stimulates cell migration and invasion by up-regulation of matrix metalloproteinases (Gupta and DuBois, 2001). PGE_2 and COX-2 overexpression also correlates with CRC risk and metastasis of CRC (Tsujii *et al.*, 1997), making this pathway relevant also in follow-up after treatment of the primary cancer. Furthermore, our observations show that the PGE_2 produced also inhibits anti-tumour immunity in primary CRC through the EP2/EP4 prostanoid receptor – cAMP – PKA – Csk pathway in effector T cells that inhibit T cell activation (Figure 1A and B) (Yaqub and Tasken, 2008; Yaqub *et al.*, 2008; Oberprieler *et al.*, 2010). In addition, our prospective studies of disease recurrence in patients with metastatic CRC that underwent liver resection of metastases showed strong correlation between the degree of Treg and PGE_2-mediated suppression of anti-tumour activity and disease recurrence, further substantiating the significance of COX-2 and PGE_2-mediated regulation of anti-tumour immune activity (K.W. Brudvik *et al.*, submitted).

Other human tumours also express high levels of COX-2 (Eberhart *et al.*, 1994; Brosens *et al.*, 2005; Steffensen *et al.*, 2007). Tumour-derived PGE_2 contributes to cancer progression among others by promoting the conversion of adaptive Treg cells into a regulatory phenotype (Baratelli *et al.*, 2005; Sharma *et al.*, 2005). Treg cells induced in the periphery have been shown to accumulate at tumour sites, where they may impair the development of effective anti-tumour immune responses (Liu *et al.*, 2007). Patients with several forms of cancer have been shown to have increased numbers of circulating and tumour-associated Treg cells compared with healthy controls. In patients with gastrointestinal malignancies, the percentage of Treg cells in peripheral blood correlates inversely with disease prognosis (de Souza and Bonorino, 2009). Moreover, depletion of Treg cells in animal models enhances anti-tumour responses (Sakaguchi *et al.*, 2001; Tanaka *et al.*, 2002).

Therapeutic targeting of the cAMP/PGE_2 pathway in disease

The activities of both type I PKA and PDE4 seem to be important for regulation of TCR-induced signalling and T cell function. Type I PKA is activated by external stimuli such as PGE2 produced by regulatory T cells (Mahic *et al.*, 2006), monocytes (Bryn *et al.*, 2006) and adrenergic agonists as well as by an intrinsic mechanism upon TCR stimulation, both of which induces cAMP production in lipid rafts and inhibits proximal T cell signalling (Abrahamsen *et al.*, 2004). However, CD28 co-stimulation leads to recruitment of a PDE4/β-arrestin complex that releases cAMP inhibition allowing a full T cell response to occur (Bjorgo *et al.*, 2010). Finally, ezrin is the essential link between cytoskeletal F-actin and a lipid raft-associated multiprotein complex including EBP50, Cbp/PAG, Csk and PKA type I that negatively regulates immune responses upon T cell activation.

As high levels of endogenous cAMP may be responsible for the anergic phenotype of Treg cells, as well as being central to their suppressive action, uncoupling of the cAMP-type I PKA-Csk pathway in responder T cells would potentially render these cells resistant to Treg cell-mediated suppression. Interestingly, pharmacological intervention that seeks to normalize the elevated cAMP levels in effector T cells has been shown to attenuate Treg cell-mediated suppression of IL-2 secretion (Bopp *et al.*, 2007). The ectoenzymes CD39 and CD73 together generate adenosine on the Treg cell surface. Loss of CD39 and inhibition of CD73 enzymatic activity as well as use of an adenosine receptor antagonist abrogated Treg cell-mediated suppressive function in the presence of 5′AMP *in vitro* and *in vivo* (Kobie *et al.*, 2006; Deaglio *et al.*, 2007; Mandapathil and Whiteside, 2011). Thus, perturbed cAMP signalling in responder T cells would potentially render them resistant to Treg cell-mediated suppression and facilitate anti-tumour immune responses. Our published data from characterization of transgenic mice perturbed in the cAMP-type I PKA-Csk pathway by T cell-specific expression of RIAD show that this intervention protects from disease (Mosenden *et al.*, 2011). Moreover, our work with characterizing PKA type I selective cAMP antagonists shows that such compounds also may effectively block cAMP-mediated immune suppression both in patent samples *ex vivo* and in animal disease models (Aandahl *et al.*, 1998; 1999;

Aukrust *et al*., 1999; Rahmouni *et al*., 2001; Holm *et al*., 2003; Nayjib *et al*., 2008; Yaqub *et al*., 2008). In contrast, preclinical studies demonstrate that cAMP-elevating agents like vasoactive intestinal peptide or PDE inhibitors can alleviate autoimmune diseases via the increase of cAMP in self-reactive effector T cells (Delgado *et al*., 2002; Aricha *et al*., 2006). Selective inhibitors of PDE4 have been proposed as novel anti-inflammatory agents for T cell-mediated diseases in that rolipram has been documented to suppress TNF- and IFN-γ production in animal models of rheumatoid arthritis and has been suggested to have chondroprotective effects (Essayan, 2001). Clinical trials with the PDE4 inhibitor piclamilast documented significant symptom relief in patients with autoimmune diseases such as rheumatoid arthritis, along with decreased levels of IL-6 and C-reactive protein (Souness *et al*., 2000). Furthermore, inhibition of PDEs can alleviate autoimmune diseases via the increase of cAMP in self-reactive effector T cells (Aricha *et al*., 2006). Lastly, PDE7 is also up-regulated post-activation, and PDE7 or PDE4/7 inhibitors may also have application in inflammatory diseases (Giembycz, 2008). In conclusion, the cAMP-type I PKA-Csk pathway emerges as a putative target for therapeutic perturbation in chronic inflammation in cancer as well as in chronic viral disease, whereas pacing the same pathway may be beneficial in autoimmune diseases.

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Conflict of interest

The authors declare no conflict of interest.

References

Aandahl EM, Aukrust P, Skalhegg BS, Muller F, Froland SS, Hansson V *et al*. (1998). Protein kinase A type I antagonist restores immune responses of T cells from HIV-infected patients. FASEB J 12: 855–862.

Aandahl EM, Aukrust P, Muller F, Hansson V, Tasken K, Froland SS (1999). Additive effects of IL-2 and protein kinase A type I antagonist on function of T cells from HIV-infected patients on HAART. AIDS 13: F109–F114.

Aandahl EM, Moretto WJ, Haslett PA, Vang T, Bryn T, Tasken K *et al*. (2002). Inhibition of antigen-specific T cell proliferation and cytokine production by protein kinase A type I. J Immunol 169: 802–808.

Aandahl EM, Michaelsson J, Moretto WJ, Hecht FM, Nixon DF (2004). Human CD4+ CD25+ regulatory T cells control T-cell responses to human immunodeficiency virus and cytomegalovirus antigens. J Virol 78: 2454–2459.

Abrahamsen H, Baillie G, Ngai J, Vang T, Nika K, Ruppelt A *et al*. (2004). TCR- and CD28-mediated recruitment of phosphodiesterase 4 to lipid rafts potentiates TCR signaling. J Immunol 173: 4847–4858.

Algrain M, Turunen O, Vaheri A, Louvard D, Arpin M (1993). Ezrin contains cytoskeleton and membrane binding domains accounting for its proposed role as a membrane-cytoskeletal linker. J Cell Biol 120: 129–139.

Aricha R, Feferman T, Souroujon MC, Fuchs S (2006). Overexpression of phosphodiesterases in experimental autoimmune myasthenia gravis: suppression of disease by a phosphodiesterase inhibitor. FASEB J 20: 374–376.

Aukrust P, Aandahl EM, Skalhegg BS, Nordoy I, Hansson V, Tasken K *et al*. (1999). Increased activation of protein kinase A type I contributes to the T cell deficiency in common variable immunodeficiency. J Immunol 162: 1178–1185.

Baillie GS, Scott JD, Houslay MD (2005). Compartmentalisation of phosphodiesterases and protein kinase A: opposites attract. FEBS Lett 579: 3264–3270.

Baratelli F, Lin Y, Zhu L, Yang SC, Heuze-Vourc'h N, Zeng G *et al*. (2005). Prostaglandin E2 induces FOXP3 gene expression and T regulatory cell function in human CD4+ T cells. J Immunol 175: 1483–1490.

Bjorgo E, Solheim SA, Abrahamsen H, Baillie GS, Brown KM, Berge T *et al*. (2010). Cross talk between phosphatidylinositol 3-kinase and cyclic AMP (cAMP)-protein kinase a signaling pathways at the level of a protein kinase B/beta-arrestin/cAMP phosphodiesterase 4 complex. Mol Cell Biol 30: 1660–1672.

Bluestone JA, Abbas AK (2003). Natural versus adaptive regulatory T cells. Nat Rev Immunol 3: 253–257.

Bopp T, Becker C, Klein M, Klein-Hessling S, Palmetshofer A, Serfling E *et al*. (2007). Cyclic adenosine monophosphate is a key component of regulatory T cell-mediated suppression. J Exp Med 204: 1303–1310.

Brdicka T, Pavlistova D, Leo A, Bruyns E, Korinek V, Angelisova P *et al*. (2000). Phosphoprotein associated with glycosphingolipid-enriched microdomains (PAG), a novel ubiquitously expressed transmembrane adaptor protein, binds the protein tyrosine kinase csk and is involved in regulation of T cell activation. J Exp Med 191: 1591–1604.

Brenchley JM, Price DA, Schacker TW, Asher TE, Silvestri G, Rao S *et al*. (2006). Microbial translocation is a cause of systemic immune activation in chronic HIV infection. Nat Med 12: 1365–1371.

Bretscher A, Edwards K, Fehon RG (2002). ERM proteins and merlin: integrators at the cell cortex. Nat Rev Mol Cell Biol 3: 586–599.

Brosens LA, Iacobuzio-Donahue CA, Keller JJ, Hustinx SR, Carvalho R, Morsink FH *et al*. (2005). Increased cyclooxygenase-2 expression in duodenal compared with colonic tissues in familial adenomatous polyposis and relationship to the -765G -> C COX-2 polymorphism. Clin Cancer Res 11: 4090–4096.

Bryn T, Mahic M, Enserink JM, Schwede F, Aandahl EM, Tasken K (2006). The cyclic AMP-Epac1-Rap1 pathway is dissociated from regulation of effector functions in monocytes but acquires immunoregulatory function in mature macrophages. J Immunol 176: 7361–7370.

Cadogan M, Dalgleish AG (2008). Pathogenesis of HIV: non-specific immune hyperactivity and its implications for vaccines. Clin Med 8: 267–271.

Carlson CR, Lygren B, Berge T, Hoshi N, Wong W, Tasken K *et al*. (2006). Delineation of type I protein kinase A-selective signaling events using an RI anchoring disruptor. J Biol Chem 281: 21535–21545.

Deaglio S, Dwyer KM, Gao W, Friedman D, Usheva A, Erat A *et al.* (2007). Adenosine generation catalyzed by CD39 and CD73 expressed on regulatory T cells mediates immune suppression. J Exp Med 204: 1257–1265.

Delgado M, Abad C, Martinez C, Juarranz MG, Arranz A, Gomariz RP *et al.* (2002). Vasoactive intestinal peptide in the immune system: potential therapeutic role in inflammatory and autoimmune diseases. J Mol Med 80: 16–24.

Douek DC, Picker LJ, Koup RA (2003). T cell dynamics in HIV-1 infection. Annu Rev Immunol 21: 265–304.

Eberhart CE, Coffey RJ, Radhika A, Giardiello FM, Ferrenbach S, DuBois RN (1994). Up-regulation of cyclooxygenase 2 gene expression in human colorectal adenomas and adenocarcinomas. Gastroenterology 107: 1183–1188.

Essayan DM (2001). Cyclic nucleotide phosphodiesterases. J Allergy Clin Immunol 108: 671–680.

Fujino H, Toyomura K, Chen X, Regan JW, Murayama T (2011). Prostaglandin E2 regulates cellular migration via induction of vascular endothelial growth factor receptor-1 in HCA-7 human colon cancer cells. Biochem Pharmacol 81: 379–387.

Fuld S, Borland G, Yarwood SJ (2005). Elevation of cyclic AMP in Jurkat T-cells provokes distinct transcriptional responses through the protein kinase A (PKA) and exchange protein activated by cyclic AMP (EPAC) pathways. Exp Cell Res 309: 161–173.

Gavin MA, Rasmussen JP, Fontenot JD, Vasta V, Manganiello VC, Beavo JA *et al.* (2007). Foxp3-dependent programme of regulatory T-cell differentiation. Nature 445: 771–775.

Gerlo S, Verdood P, Hooghe-Peters EL, Kooijman R (2006). Multiple cAMP-induced signaling cascades regulate prolactin expression in T cells. Cell Mol Life Sci 63: 92–99.

Giembycz MA (2008). Can the anti-inflammatory potential of PDE4 inhibitors be realized: guarded optimism or wishful thinking? Br J Pharmacol 155: 288–290.

Giorgi JV, Lyles RH, Matud JL, Yamashita TE, Mellors JW, Hultin LE *et al.* (2002). Predictive value of immunologic and virologic markers after long or short duration of HIV-1 infection. J Acquir Immune Defic Syndr 29: 346–355.

Gloerich M, Bos JL (2010). Epac: defining a new mechanism for cAMP action. Annu Rev Pharmacol Toxicol 50: 355–375.

Gupta RA, DuBois RN (2001). Colorectal cancer prevention and treatment by inhibition of cyclooxygenase-2. Nat Rev Cancer 1: 11–21.

Hanoune J, Defer N (2001). Regulation and role of adenylyl cyclase isoforms. Annu Rev Pharmacol Toxicol 41: 145–174.

Hazenberg MD, Otto SA, van Benthem BH, Roos MT, Coutinho RA, Lange JM *et al.* (2003). Persistent immune activation in HIV-1 infection is associated with progression to AIDS. AIDS 17: 1881–1888.

Holm AM, Aukrust P, Aandahl EM, Muller F, Tasken K, Froland SS (2003). Impaired secretion of IL-10 by T cells from patients with common variable immunodeficiency–involvement of protein kinase A type I. J Immunol 170: 5772–5777.

Holm M, Pettersen FO, Kvale D (2008). PD-1 predicts CD4 loss rate in chronic HIV-1 infection better than HIV RNA and CD38 but not in cryopreserved samples. Curr HIV Res 6: 49–58.

Huang B, Zhao J, Lei Z, Shen S, Li D, Shen GX *et al.* (2009). miR-142-3p restricts cAMP production in CD4+. EMBO Rep 10: 180–185.

Jarnaess E, Ruppelt A, Stokka AJ, Lygren B, Scott JD, Tasken K (2008). Dual specificity A-kinase anchoring proteins (AKAPs) contain an additional binding region that enhances targeting of protein kinase A type I. J Biol Chem 283: 33708–33718.

Johansson CC, Bryn T, Aandahl EM, Areklett MA, Aukrust P, Tasken K *et al.* (2004). Treatment with type-2 selective and non-selective cyclooxygenase inhibitors improves T-cell proliferation in HIV-infected patients on highly active antiretroviral therapy. AIDS 18: 951–952.

Kammer GM (1988). The adenylate cyclase-cAMP-protein kinase A pathway and regulation of the immune response. Immunol Today 9: 222–229.

Kaupp UB, Seifert R (2002). Cyclic nucleotide-gated ion channels. Physiol Rev 82: 769–824.

Kawabuchi M, Satomi Y, Takao T, Shimonishi Y, Nada S, Nagai K *et al.* (2000). Transmembrane phosphoprotein Cbp regulates the activities of Src-family tyrosine kinases. Nature 404: 999–1003.

Kobie JJ, Shah PR, Yang L, Rebhahn JA, Fowell DJ, Mosmann TR (2006). T regulatory and primed uncommitted CD4 T cells express CD73, which suppresses effector CD4 T cells by converting 5′-adenosine monophosphate to adenosine. J Immunol 177: 6780–6786.

Kraus S, Arber N (2011). Cancer: do aspirin and other NSAIDs protect against colorectal cancer? Nat Rev Gastroenterol Hepatol 8: 125–126.

Kvale D, Aaberge IS, Froland SS (2002). Predictive value of immunologic parameters and HIV RNA in relation to humoral pneumococcal polysaccharide vaccine responses in patients with HIV infection. Eur J Clin Microbiol Infect Dis 21: 688–690.

Kvale D, Ormaasen V, Kran AM, Johansson CC, Aukrust P, Aandahl EM *et al.* (2006). Immune modulatory effects of cyclooxygenase type 2 inhibitors in HIV patients on combination antiretroviral treatment. AIDS 20: 813–820.

Levy FO, Rasmussen AM, Tasken K, Skalhegg BS, Huitfeldt HS, Funderud S *et al.* (1996). Cyclic AMP-dependent protein kinase (cAK) in human B cells: co-localization of type I cAK (RI alpha 2 C2) with the antigen receptor during anti-immunoglobulin-induced B cell activation. Eur J Immunol 26: 1290–1296.

Liu VC, Wong LY, Jang T, Shah AH, Park I, Yang X *et al.* (2007). Tumor evasion of the immune system by converting CD4+. J Immunol 178: 2883–2892.

Loza MJ, Penn RB (2010). Regulation of T cells in airway disease by beta-agonist. Front Biosci (Schol Ed) 2: 969–979.

Mahic M, Yaqub S, Johansson CC, Tasken K, Aandahl EM (2006). FOXP3+CD4+CD25+ adaptive regulatory T cells express cyclooxygenase-2 and suppress effector T cells by a prostaglandin E2-dependent mechanism. J Immunol 177: 246–254.

Mandapathil M, Whiteside TL (2011). Targeting human inducible regulatory T cells (Tr1) in patients with cancer: blocking of adenosine-prostaglandin E cooperation. Expert Opin Biol Ther 11: 1203–1214.

Mosenden R, Tasken K (2011). Cyclic AMP-mediated immune regulation–overview of mechanisms of action in T cells. Cell Signal 23: 1009–1016.

Mosenden R, Singh P, Cornez I, Heglind M, Ruppelt A, Moutschen M *et al.* (2011). Mice with disrupted type I protein

kinase A anchoring in T cells resist retrovirus-induced immunodeficiency. J Immunol 186: 5119–5130.

Nayjib B, Zeddou M, Drion P, Boniver J, Tasken K, Rahmouni S *et al*. (2008). In vivo administration of a PKA type I inhibitor (Rp-8-Br-cAMPS) restores T-cell responses in retrovirus-infected mice. Open Immunol J 1: 20–24.

Ng WF, Duggan PJ, Ponchel F, Matarese G, Lombardi G, Edwards AD *et al*. (2001). Human CD4(+)CD25(+) cells: a naturally occurring population of regulatory T cells. Blood 98: 2736–2744.

Oberprieler NG, Lemeer S, Kalland ME, Torgersen KM, Heck AJ, Tasken K (2010). High-resolution mapping of prostaglandin E2-dependent signaling networks identifies a constitutively active PKA signaling node in CD8+CD45RO+ T cells. Blood 116: 2253–2265.

Pettersen FO, Tasken K, Kvale D (2010). Combined Env- and Gag-specific T cell responses in relation to programmed death-1 receptor and CD4 T cell loss rates in human immunodeficiency virus-1 infection. Clin Exp Immunol 161: 315–323.

Pettersen FO, Torheim EA, Dahm AE, Aaberge IS, Lind A, Holm M *et al*. (2011). An exploratory trial of cyclooxygenase type 2 inhibitor in HIV-1 infection: downregulated immune activation and improved T cell-dependent vaccine responses. J Virol 85: 6557–6566.

Psaty BM, Potter JD (2006). Risks and Benefits of Celecoxib to Prevent Recurrent Adenomas. NEJM 355: 950–952.

Rahmouni S, Anadahl EM, Trebak M, Boniver J, Tasken K, Moutschen M (2001). Increased cAMP levels and protein kinase (PKA) type I activation in CD4+ T cells and B cells contribute to the retrovirus-induced immunodeficiency of mice (MAIDS). A useful in vivo model for drug testing in PKA type I-induced immunodeficiency. FASEB J 15: 1466–1468.

Rodriguez B, Sethi AK, Cheruvu VK, Mackay W, Bosch RJ, Kitahata M *et al*. (2006). Predictive value of plasma HIV RNA level on rate of CD4 T-cell decline in untreated HIV infection. JAMA 296: 1498–1506.

Ruppelt A, Mosenden R, Gronholm M, Aandahl EM, Tobin D, Carlson CR *et al*. (2007). Inhibition of T cell activation by cyclic adenosine 5′-monophosphate requires lipid raft targeting of protein kinase A type I by the A-kinase anchoring protein ezrin. J Immunol 179: 5159–5168.

Sakaguchi S, Sakaguchi N, Shimizu J, Yamazaki S, Sakihama T, Itoh M *et al*. (2001). Immunologic tolerance maintained by CD25+ CD4+ regulatory T cells: their common role in controlling autoimmunity, tumor immunity, and transplantation tolerance. Immunol Rev 182: 18–32.

Sakaguchi S, Ono M, Setoguchi R, Yagi H, Hori S, Fehervari Z *et al*. (2006). Foxp3+ CD25+ CD4+ natural regulatory T cells in dominant self-tolerance and autoimmune disease. Immunol Rev 212: 8–27.

Sharma S, Yang SC, Zhu L, Reckamp K, Gardner B, Baratelli F *et al*. (2005). Tumor cyclooxygenase-2/prostaglandin E2-dependent promotion of FOXP3 expression and CD4+ CD25+ T regulatory cell activities in lung cancer. Cancer Res 65: 5211–5220.

Sheng H, Shao J, Washington MK, DuBois RN (2001). Prostaglandin E2 increases growth and motility of colorectal carcinoma cells. J Biol Chem 276: 18075–18081.

Skalhegg BS, Landmark BF, Doskeland SO, Hansson V, Lea T, Jahnsen T (1992). Cyclic AMP-dependent protein kinase type I mediates the inhibitory effects of 3′,5′-cyclic adenosine monophosphate on cell replication in human T lymphocytes. J Biol Chem 267: 15707–15714.

Skalhegg BS, Tasken K, Hansson V, Huitfeldt HS, Jahnsen T, Lea T (1994). Location of cAMP-dependent protein kinase type I with the TCR-CD3 complex. Science 263: 84–87.

Souness JE, Aldous D, Sargent C (2000). Immunosuppressive and anti-inflammatory effects of cyclic AMP phosphodiesterase (PDE) type 4 inhibitors. Immunopharmacology 47: 127–162.

de Souza AP, Bonorino C (2009). Tumor immunosuppressive environment: effects on tumor-specific and nontumor antigen immune responses. Expert Rev Anticancer Ther 9: 1317–1332.

Steffensen KD, Waldstrom M, Jeppesen U, Jakobsen E, Brandslund I, Jakobsen A (2007). The prognostic importance of cyclooxygenase 2 and HER2 expression in epithelial ovarian cancer. Int J Gynecol Cancer 17: 798–807.

Stokka AJ, Mosenden R, Ruppelt A, Lygren B, Tasken K (2010). The adaptor protein EBP50 is important for localization of the protein kinase A-Ezrin complex in T-cells and the immunomodulating effect of cAMP. Biochem J 425: 381–388.

Takeuchi S, Takayama Y, Ogawa A, Tamura K, Okada M (2000). Transmembrane phosphoprotein Cbp positively regulates the activity of the carboxyl-terminal Src kinase, Csk. J Biol Chem 275: 29183–29186.

Tanaka H, Tanaka J, Kjaergaard J, Shu S (2002). Depletion of CD4+ CD25+ regulatory cells augments the generation of specific immune T cells in tumor-draining lymph nodes. J Immunother 25: 207–217.

Torgersen KM, Vaage JT, Levy FO, Hansson V, Rolstad B, Tasken K (1997). Selective activation of cAMP-dependent protein kinase type I inhibits rat natural killer cell cytotoxicity. J Biol Chem 272: 5495–5500.

Torgersen KM, Vang T, Abrahamsen H, Yaqub S, Horejsi V, Schraven B *et al*. (2001). Release from tonic inhibition of T cell activation through transient displacement of C-terminal Src kinase (Csk) from lipid rafts. J Biol Chem 276: 29313–29318.

Torgersen KM, Vang T, Abrahamsen H, Yaqub S, Tasken K (2002). Molecular mechanisms for protein kinase A-mediated modulation of immune function. Cell Signal 14: 1–9.

Torgersen KM, Aandahl EM, Tasken K (2008). Molecular architecture of signal complexes regulating immune cell function. Handb Exp Pharmacol 186: 327–363.

Torheim EA, Ndhlovu LC, Pettersen FO, Larsen TL, Jha AR, Torgersen KM *et al*. (2009). Interleukin-10-secreting T cells define a suppressive subset within the HIV-1-specific T-cell population. Eur J Immunol 39: 1280–1287.

Treves AJ, Ligumski M, Fuks Z, Haimovitz A, Rachmilewitz D (1982). Prostaglandin E2 secretion by subsets of human peripheral blood monocytes. Immunopharmacology 4: 267–276.

Tsujii M, Kawano S, DuBois RN (1997). Cyclooxygenase-2 expression in human colon cancer cells increases metastatic potential. Proc Natl Acad Sci USA 94: 3336–3340.

Tsukita S, Oishi K, Sato N, Sagara J, Kawai A, Tsukita S (1994). ERM family members as molecular linkers between the cell surface glycoprotein CD44 and actin-based cytoskeletons. J Cell Biol 126: 391–401.

Vang T, Torgersen KM, Sundvold V, Saxena M, Levy FO, Skalhegg BS *et al*. (2001). Activation of the COOH-terminal Src kinase (Csk) by cAMP-dependent protein kinase inhibits signaling through the T cell receptor. J Exp Med 193: 497–507.

Vang T, Abrahamsen H, Myklebust S, Horejsi V, Tasken K (2003). Combined spatial and enzymatic regulation of Csk by cAMP and protein kinase a inhibits T cell receptor signaling. J Biol Chem 278: 17597–17600.

Willoughby D, Cooper DM (2007). Organization and Ca2+ regulation of adenylyl cyclases in cAMP microdomains. Physiol Rev 87: 965–1010.

Yaqub S, Tasken K (2008). Role for the cAMP-protein kinase A signaling pathway in suppression of antitumor immune responses by regulatory T cells. Crit Rev Oncog 14: 57–77.

Yaqub S, Henjum K, Mahic M, Jahnsen FL, Aandahl EM, Bjornbeth BA *et al*. (2008). Regulatory T cells in colorectal cancer patients suppress anti-tumor immune activity in a COX-2 dependent manner. Cancer Immunol Immunother 57: 813–821.

Yonemura S, Hirao M, Doi Y, Takahashi N, Kondo T, Tsukita S *et al*. (1998). Ezrin/radixin/moesin (ERM) proteins bind to a positively charged amino acid cluster in the juxta-membrane cytoplasmic domain of CD44, CD43, and ICAM-2. J Cell Biol 140: 885–895.