Cutting Edge: A-Kinase Anchor Proteins Are Involved in Maintaining Resting T Cells in an Inactive State

Richard O. Williams

*J Immunol* 2002; 168:5392-5396; doi: 10.4049/jimmunol.168.11.5392
http://www.jimmunol.org/content/168/11/5392

References

This article cites 22 articles, 9 of which you can access for free at:
http://www.jimmunol.org/content/168/11/5392.full#ref-list-1

Why *The JI*? Submit online.

- **Rapid Reviews! 30 days** from submission to initial decision
- **No Triage!** Every submission reviewed by practicing scientists
- **Fast Publication!** 4 weeks from acceptance to publication

*average

Subscription

Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

Permissions

Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts

Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
A-kinase anchor proteins (AKAPs) target protein kinase A (PKA) to different subcellular locations and are thought to play important roles in the cAMP signaling pathway. The aims of this study were to determine whether T cells express AKAPs and, if so, to establish their physiological significance. CD4⁺ T cells were found to express eight AKAPs. Disruption of the AKAP-PKA interaction caused high levels of IL-2, IL-4, IL-5, and IFN-γ production in the absence of stimulation via CD3ε and CD28 molecules. Disruption of the AKAP-PKA interaction acted synergistically with suboptimal doses of Ag in boosting proliferative responses of T cells. Finally, disruption of the AKAP-PKA interaction rendered T cells insensitive to cAMP-elevating agents. It was concluded that AKAPs, through their association with PKA, are involved in maintaining T cell homeostasis and in regulating the sensitivity of T cells to incoming cAMP signals. The Journal of Immunology, 2002, 168: 5392–5396.

Elevated intracellular levels of cAMP in lymphocytes have a negative regulatory effect on proliferation and cytokine expression; therefore, the cAMP signaling pathway represents a potentially important inhibitory influence on T cell activity (1). Levels of cAMP are governed by two families of enzymes: adenylate cyclase (which generates cAMP) and phosphodiesterase (which degrades cAMP). The accumulation of cAMP beyond a threshold level results in activation of protein kinase A (PKA), an enzyme with broad substrate specificity. In addition, there is now a growing body of evidence suggesting that a group of proteins, known as A-kinase anchor proteins (AKAPs), plays an important role in regulating the activity of PKA by targeting the enzyme to different subcellular compartments (2). Thus, all of the AKAPs possess not only a subcellular targeting motif but also a motif that binds the type II regulatory subunit (RII) of the PKA holoenzyme, thereby enabling compartmentalization of PKA (3). This compartmentalization of PKA by AKAP has two potentially important consequences. First, by anchoring PKA close to the site of cAMP generation, AKAPs may increase sensitivity to incoming cAMP signals. Second, by targeting PKA to different subcellular organelles, AKAPs may influence the enzyme substrate specificity of PKA (3). Therefore, a model has been proposed in which AKAPs play crucial roles in cAMP signaling by integrating upstream activators and downstream targets of PKA (4).

The objectives of this study were, first, to determine whether murine T cells express AKAP and, second, to establish the physiological significance of the AKAP-PKA interaction using an inhibitor of AKAP-PKA binding. The findings reveal that the interaction between AKAP and PKA plays a major role in maintaining T cells in an inactive state.

Materials and Methods

Mice

BALB/c mice were purchased from Harlan Olac (Bicester, U.K.). HNT TCR-transgenic mice, which express a TCR specific for an influenza hemagglutinin peptide (126–138) (5) were bred at the Kennedy Institute (London, U.K.) on a BALB/c background from founder stock, kindly provided by Dr. R. Liblau (Laboratory of Cellular Immunology, Paris, France). All mice were used at 8–12 wk of age.

RII overlay assay

A modification of the RII overlay assay, as described by Carr and Scott (6), was used in this study to detect AKAPs in T cell lysates. CD4⁺ T cells were isolated from the spleens of BALB/c mice by magnetic cell sorting (Miltenyi Biotec, Bergisch Gladbach, Germany) according to the manufacturer’s instructions. Purity was >90%, as assessed by FACS analysis.
The cells were then lysed for 15 min on ice in lysis buffer (20 mM HEPES (pH 7.4), 20 mM NaCl, 1 mM EDTA, 0.2 mM DTT, 1% v/v Triton X-100, and 1 mM PMSE). Cell lysates were separated by gel electrophoresis using NuPAGE Novex 3–8% Tris-acetate gels (Invitrogen, Groningen, The Netherlands) and transferred to nitrocellulose membranes. The membranes were then blocked with 5% nonfat milk powder and incubated with recombinant RII, followed by rabbit anti-RII Ab and HRP-conjugated anti-rabbit IgG. In addition to the RII overlay assay, AKAP150 was further identified using a standard Western blotting procedure using rabbit anti-AKAP150 Ab. Recombinant RII, anti-RII Ab, and anti-AKAP150 Ab were all generously provided by Dr. C. Loh and Dr. Y. Lai (ICOS, Bothell, WA).

Peptides
HA (126–138) peptide (HNTNGVTAACSHE) was synthesized by the Advanced Biotechnology Center (Imperial College, London, U.K.).

St-H31 is a stearated form of the peptide (493–515) Ht31 (human thyroid AKAP) that inhibits the interaction of the RII subunit of PKA with AKAP (7–10). The presence of the stearated moiety renders Ht31 cell permeant (7). St-H31P is a control peptide in which two isoleucine residues have been replaced by proline residues, thereby blocking its ability to disrupt the AKAP-PKA interaction (7). St-H31 (N-stearate-DLIEEAAS RIVDAVIEQVKAAGAY) and St-H31P (N-stearate-DLIEEAASRPV DAVPEQVKAAGAY) were both purchased from Promega (Madison, WI).

T cell cultures
Spleen cells from HNT TCR-transgenic mice were cultured at a density of 2 × 10^6/ml in 96-well plates in RPMI 1640 containing FCS (10% v/v), 2-ME (20 μM), l-glutamine (1% w/v), penicillin (100 U/ml), and streptomycin (100 μg/ml). HA (126–138) peptide was then added to the cells, followed 1–2 h later by St-H31, St-H31P, or vehicle (50 mM Tris-HCl, pH 7.5). Cultures were assayed in triplicate after 24 h for IL-2 and after 72 h for IL-4, IL-5, and IFN-γ. To determine the rate of T cell proliferation, triplicate cultures were pulsed after 48 h with [3H]thymidine and cultured for an additional 16 h. Cells were then harvested and assessed for incorporation of radioactivity.

To analyze the effect of disrupting the AKAP-PKA interaction in purified populations of T cells, CD4^+ T cells were isolated from the spleens of BALB/c mice by magnetic cell sorting, then cultured alone or with St-H31 or St-H31P. Alternatively, T cells were stimulated with plate-bound anti-CD3e (5 μg/ml) and soluble anti-CD28 (10 μg/ml). Anti-CD3e and anti-CD28 were purchased from AMS Biotechnology (Abingdon, U.K.).

Measurement of cytokines
To measure secreted cytokines, 96-well ELISA plates were coated with the respective capture Ab (purchased from AMS Biotechnology), blocked with BSA (2% w/v), and then incubated with culture supernatants. After washing, bound cytokines were detected using biotinylated detect Abs (AMS Biotechnology) followed by Europium-conjugated avidin and enhancement solution (Wallac, Turku, Finland). Fluorescence was then measured with a time-resolved fluorometer (Victor 1420; PerkinElmer, Beaconsfield, U.K.). A standard curve was generated using known concentrations of the appropriate recombinant cytokine (AMS Biotechnology).

Results and Discussion
RII overlay assay reveals multiple AKAPs in CD4^+ T cells
The RII overlay assay takes advantage of the fact that AKAPs retain the ability to bind the RII subunit of PKA after gel electrophoresis under denaturing conditions, followed by immobilization on nitrocellulose membranes (11). To determine whether T cells express AKAPs, CD4^+ T cells were isolated from the spleens of unimmunized BALB/c mice by MACS separation. Cell lysates were then prepared, separated by SDS-PAGE, and transferred to nitrocellulose membrane for the detection of AKAPs. Using RII as a probe, at least eight AKAPs were detected, with apparent molecular masses of 60, 75, 95, 120, 165, 190, 245, and 275 kDa (Fig. 1, lanes 1 and 2). Preincubation of RII with excess St-H31 peptide, which competes with AKAP for binding to RII, blocked the binding of RII to the AKAPs present in the T cell lysates (Fig. 1, lanes 3 and 4). This suggests that all of the eight bands detected in the overlay assay were true AKAPs. In a very recently published study, Schillace et al. detected at least six AKAPs in T cell-enriched human PBMCs and in Jurkat cells (12). This present study and that of Schillace et al. (12) represent the first reports of AKAP expression in murine and human T cells, respectively.

One of the AKAPs detected in the RII overlay assay was identified as AKAP150, the murine ortholog of human AKAP79 (Fig. 1, lanes 5 and 6). This is consistent with the observation that AKAP79 was also detected in human T cells (12). The subcellular targeting domains of human AKAP79 and murine AKAP150 consist of a sequence of three N-terminal polybasic residues that bind to acidic phospholipids of the inner leaflet of the cell membrane (13). This is significant because adenylate cyclase is also located in the cell membrane and therefore AKAP79 and AKAP150 are thought to be important in determining cellular sensitivity to cAMP-elevating agents by targeting PKA to the site of cAMP generation. This was confirmed in a study in which transfection of HEK293 cells (which are AKAP deficient) with the gene encoding AKAP75 (the bovine ortholog of AKAP79 and AKAP150) caused translocation of PKA from the cytosol to the cell membrane and dramatically up-regulated sensitivity to forskolin, an activator of adenylate cyclase (14). AKAP79 and AKAP150 also contain binding sites for at least two other signaling molecules known to be of major importance in the activation of T cells, protein kinase C and calcineurin (15, 16), although the physiological significance of these interactions remains to be established.

Disruption of the AKAP-PKA interaction induces cytokine production
The effect was studied of disrupting the AKAP-PKA interaction on the production of cytokines using St-H31, which competes with AKAP for binding to PKA. Treatment of spleen cells with St-H31 caused high levels of production of IL-2, IL-4, IL-5, and IFN-γ (Fig. 2). To exclude the possibility that St-H31 was acting via...
stimulation of APC activity, CD4⁺ T cells were isolated from the spleens of BALB/c mice and cultured alone, with St-Ht31 or St-Ht31P. For comparison, CD4⁺ T cells were stimulated with plate-bound anti-CD3ε and soluble anti-CD28 molecules (Fig. 3). Although this study focused on CD4⁺ T cells, treatment of CD8⁺ T cells with St-Ht31 was also found to stimulate cytokine release (data not shown).

Disruption of the AKAP-PKA interaction synergizes with Ag in stimulation of T cell proliferation

The activation of T cells via the TCR and costimulatory molecules normally results in both cytokine release and proliferation. This study has so far demonstrated that disruption of the AKAP-PKA interaction causes the release of cytokines, but it is not clear whether this involves an increase in the rate of T cell proliferation. To address this question, spleen cells were cultured in the presence of St-Ht31 or the control peptide St-Ht31P and a proliferation assay was conducted. Incubation with St-Ht31 caused a modest (2- to 3-fold) increase in [³H]thymidine incorporation (data not shown). Next, the question was addressed of whether St-Ht31 synergizes with Ag in the stimulation of T cell proliferation. A proliferation assay was conducted using T cells from HNT TCR-transgenic mice, which recognize a peptide of influenza hemagglutinin. Spleen cells from HNT TCR-transgenic mice were cultured with increasing concentrations of Ag in the presence of St-Ht31 or the control peptide, St-Ht31P. As before, St-Ht31 alone caused a modest increase in T cell proliferation. However, St-Ht31 was found to synergize with suboptimal concentrations of Ag in the stimulation of T cell proliferation (Fig. 4). Thus, Ag alone at a concentration of 0.125 μg/ml failed to stimulate proliferation over the background level. However, in combination with St-Ht31, the same Ag concentration caused a 7-fold increase in [³H]thymidine incorporation.

This increase in proliferation is likely to be due to the increased production of IL-2, which is often the limiting factor in the development of immune responses (17). Indeed, the lack of IL-2 production is widely used as a marker of anergic or unresponsive T cells (18), and anergy can be broken by the addition of exogenous Ags to anergic T cells (19). The fact that St-Ht31 is able to synergize with suboptimal concentrations of Ag may have important implications for vaccine development, because many vaccines (e.g., against parasites or tumor Ags) show poor efficacy due to a failure to elicit robust T cell responses. Therefore, it may be possible to use
that both type I and type II PKA play critical roles in mediating the inhibitory effects of cAMP on downstream Ag-driven signaling events (22). Type II PKA comprises the majority of anchored PKA and, by implication, treatment of cells with St-Ht31 predominantly influences the activity of type II PKA. However, AKAPs may also bind type I PKA, and it is not possible, on the basis of this study, to conclude that it is the type II PKA isozyme that is involved in regulating the activity of resting T cells.

Conclusions
This study has demonstrated the importance of AKAPs in regulating the activity of T cells and in determining their sensitivity to incoming cAMP signals, and it is concluded that AKAPs contribute to the maintenance of T cell homeostasis. However, important questions remain to be addressed. For example, it will be important to establish whether the interaction between AKAP and PKA inhibits T cell activity by rendering the cAMP/PKA pathway constitutively active, or whether it sensitizes the cells to very low levels of endogenous cAMP-elevating agents. Another priority will be to identify which of the AKAPs are responsible for regulating T cell activity and which T cell activation pathways are inhibited by the AKAP-PKA interaction in T cells. Notwithstanding these questions, the finding that disruption of the AKAP-PKA interaction stimulates T cell cytokine production, desensitizes T cells to cAMP-elevating agents, and acts in synergy with suboptimal doses of Ag in boosting proliferative responses may have important implications in the development of effective vaccines against poorly immunogenic Ags.

Acknowledgments
I greatly appreciate the assistance provided by Salman Ahmed. I am also indebted to Roland Liblau of the Laboratory of Cellular Immunology for providing the HNT TCR-transgenic mice and to Yvonne Lai and Christine Loh of ICOS for providing recombinant RII and Abs to RII and AKAP150.

References


