Cyclosporine-Resistant, Rab27a-Independent Mobilization of Intracellular Preformed CD40 Ligand Mediates Antigen-Specific T Cell Help In Vitro

Yoshinobu Koguchi, Jennifer L. Gardell, Timothy J. Thauland and David C. Parker

*J Immunol* 2011; 187:626-634; Prepublished online 15 June 2011;
doi: 10.4049/jimmunol.1004083
http://www.jimmunol.org/content/187/2/626

Supplementary Material

http://www.jimmunol.org/content/suppl/2011/06/15/jimmunol.1004083.DC1

References

This article cites 63 articles, 25 of which you can access for free at:
http://www.jimmunol.org/content/187/2/626.full#ref-list-1

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
Cyclosporine-Resistant, Rab27a-Independent Mobilization of Intracellular Preformed CD40 Ligand Mediates Antigen-Specific T Cell Help In Vitro

Yoshinobu Koguchi, Jennifer L. Gardell, Timothy J. Thauland, and David C. Parker

CD40L is critically important for the initiation and maintenance of adaptive immune responses. It is generally thought that CD40L expression in CD4+ T cells is regulated transcriptionally and made from new mRNA following Ag recognition. However, recent studies with two-photon microscopy revealed that most cognate interactions between effector CD4+ T cells and APCs are too short for de novo synthesis of CD40L. Given that effector and memory CD4+ T cells store preformed CD40L (pCD40L) in lysosomal compartments and that pCD40L comes to the cell surface within minutes of antigenic stimulation, we and others have proposed that pCD40L might mediate T cell-dependent activation of cognate APCs during brief encounters in vivo. However, it has not been shown that this relatively small amount of pCD40L is sufficient to activate APCs, owing to the difficulty of separating the effects of pCD40L from those of de novo CD40L and other cytokines in vitro. In this study, we show that pCD40L surface mobilization is resistant to cyclosporine or FK506 treatment, while de novo CD40L and cytokine expression are completely inhibited. These drugs thus provide a tool to dissect the role of pCD40L in APC activation. We find that pCD40L mediates selective activation of cognate but not bystander APCs in vitro and that mobilization of pCD40L does not depend on Rab27a, which is required for mobilization of lytic granules. Therefore, effector CD4+ T cells deliver pCD40L specifically to APCs on the same time scale as the lethal hit of CTLs but with distinct molecular machinery. The Journal of Immunology, 2011, 187: 626–634.

Department of Molecular Microbiology and Immunology, Oregon Health & Science University, Portland, OR 97239

Received for publication December 16, 2010. Accepted for publication May 3, 2011.

This work was supported in part by National Institutes of Health Grants AI050823, AI070934, and AI077032 (to D.C.P.). J.L.G. and T.J.T. have been supported as trainees on an institutional training grant from the National Institutes of Health (T32 AI078903).

Address correspondence and reprint requests to Dr. David C. Parker, Department of Molecular Microbiology and Immunology, Oregon Health & Science University, 3181 SW Sam Jackson Park Road, L220, Portland, OR 97239. E-mail address: parkerd@ohsu.edu

The online version of this article contains supplemental material.

Abbreviations used in this article: BMDC, bone marrow-derived dendritic cell; CsA, cyclosporin A; DC, dendritic cell; dLN, draining lymph node; FaL, Faig ligand; GC, germinal center; LCMV, lymphocytic choriomeningitis virus; pCD40L, preformed CD40L; pFasL, preformed FasL; WT, wild-type.

Copyright © 2011 by The American Association of Immunologists, Inc. 0022-1767/11/$16.00

www.jimmunol.org/cgi/doi/10.4049/jimmunol.1004083

Mice

Mice were housed under specific pathogen-free conditions. These studies were approved by the Institutional Animal Care and Use Committee at Oregon Health & Science University. BALB/c, C57BL/6, C.B-17 (Igh(-)), Cd40lg(-/-), Cd45.1 congenic, and DO11.10 mice were obtained from The Jackson Laboratory (Bar Harbor, ME). Cd40lg(-/-) DO11.10 mice were bred in-house. DO11.10 Rag2(-/-) and BALB/c nude mice were obtained from Taconic Farms (Germantown, NY). Ashen mice on a C57BL/6 background were obtained from Dr. Miguel Seabra (Imperial College London, London, U.K.). SMARTA mice, which have a transgenic TCR specific for a lymphocytic choriomeningitis virus (LCMV) epitope (19), were obtained from Dr. J. Lindsay Whitton (The Scripps Research Institute). Ashen-/- or ashen/ashen SMARTA mice were bred in-house. Cd40lg(-/-) spleens were provided by Dr. David Hinrichs (Portland Veterans Affairs Medical Center, Portland, OR).
Abs and reagents

PE-Cy7-anti–Fas was purchased from BD Biosciences (San Jose, CA). FITC-PNA was from Vector Laboratories (Burlingame, CA). All other Abs for flow cytometry were purchased from eBioscience (San Diego, CA). Recombinant cytokines were purchased from PeproTech (Rocky Hill, NJ). Anti–IL-4 was from Bio X Cell (West Lebanon, NH). EasySep mouse CD4+ T cell enrichment and B cell enrichment kits were from StemCell Technologies (Vancouver, BC, Canada). Papain was from Calbiochem (San Diego, CA). Endotoxin-free OVA protein was from Profos (Regensburg, Germany). OVA323–339 Peptide was from AnaSpec (Fremont, CA). LCMV peptide gp67 was from New England Peptide (Gardner, MA). LPS (L6761), PMA, ionomycin, CsA, and FK506 were from Sigma-Aldrich (St. Louis, MO).

In vitro-generated Th1 cells

In vitro-generated Th1 cells were prepared by culturing spleen cells from DO11.10 mice in the presence of 1 μM antigenic peptide (OVA323–339) for 4 d in the presence of 1 ng/ml IL-12 and 10 μg/ml anti–IL-4. To prepare ash/+ and ash/ash polyclonal Th1 cells, purified CD4+ T cells were incubated with mouse T-Activator CD3/CD28 beads (Invitrogen, Carlsbad, CA) at a 1:1 ratio for 4 d in Th1 conditions.

In vivo-generated effector CD4+ T cells

In vivo-generated effector CD4+ T cells were obtained from the draining lymph nodes (dLNs) of BALB/c nu/nu recipients that had received 5 × 10^5 naive purified CD4+ T cells from DO11.10 Ragu2/-/- mice followed by s.c. immunization with OVA protein (50 μg) plus papain (50 μg) (20). For Fig. 7, Ag-specific in vivo Th1 cells were recovered on day 8 postinfection from LCMV (i.p. infection with 2 × 10^5 PFU LCMV Armstrong 53b)-

FIGURE 1. Calcineurin inhibitors block de novo CD40L expression and cytokine secretion but not surface mobilization of pCD40L. A, Levels of CD40L mobilization during a 30-min incubation (left, preformed CD40L) and a 120-min incubation (right, preformed plus de novo CD40L) upon PMA plus ionomycin stimulation. B, The effect of CsA (left) or FK506 (right) on the levels of CD40L mobilization upon 30- or 120-min PMA plus ionomycin stimulation. C, WT or Cd40lg-/- Th1 cells were incubated with or without PMA plus ionomycin overnight in the presence or absence of CsA or FK506. IFN-γ production was measured by ELISA with a detection limit of <0.06 ng/ml. Each bar represents the mean ± SD for triplicates. Data are representative of three independent experiments (A, B) or at least five independent experiments (C). N.D., not detected.

FIGURE 2. pCD40L in Th1 cells is sufficient to mediate selective activation of cognate B cells. For the in vitro T helper assay, CsA-pretreated Th1 cells were mixed with cognate (peptide-pulsed) and bystander (unpulsed) spleen cells for 18 h (A) or 2 d (B). As a control, Cd40lg-/- spleen cells were used. The levels of CD86, MHC class II, ICAM-1, CD62L (A), and IL-21R (B) in control, bystander, and cognate B cells are shown. Data are representative of five independent experiments.
flow cytometry

The surface mobilization assay was described previously (7). Briefly, in the surface mobilization assay, fluorochrome-labeled anti-CD40L mAb is included in the culture during the activation of cells at 37°C. Compared to the “snap shot” nature of conventional staining at 4°C after completion of stimulation, the mobilization assay captures CD40L that has been delivered to the cell surface during stimulation while blocking CD40-dependent internalization, thereby providing the “long exposure” view of CD40L expression. By limiting the stimulation period to 30 min, we were able to exclude the expression of de novo CD40L as shown previously (7).

Data were obtained with an LSR II (BD Biosciences) and analyzed with FlowJo software (Tree Star, Ashland, OR).

In vitro and ex vivo T helper assay for B cells

In vitro Th1 cells and in vivo effector CD4+ T cells were generated as described above. Wild-type (WT) or Cd40lg-/- spleen cells were differentially labeled with CFSE (21) or the CellTrace Violet dye (Invitrogen) to distinguish cognate (peptide-pulsed) and bystander (unpulsed) populations. Antigenic peptide was pulsed at 1 μM concentration at 37°C for 2 h followed by extensive washes. WT or Cd40lg-/- in vitro-generated Th1 or in vivo-generated effector CD4+ T cells were pretreated with 1 μM CsA at 37°C for 1 h and coinubated with a mixture of cognate and bystander spleen cells from WT or Cd40-/- mice in the presence of CsA throughout the assay. After the indicated hours of incubation, B cell activation was evaluated by staining for CD86, MHC class II, ICAM-1, CD62L, and IL-21 receptor. For analysis of cell division, cognate and bystander B cells were labeled with the same concentration of CFSE and were distinguished by IgM allotype (IgMa versus IgM b), and cell division was measured by CFSE dilution. For analysis of differentiation, cognate and bystander B cells were labeled with the same concentration of CFSE and were distinguished by the CD45 congenic marker (CD45.1 versus CD45.2). The T helper assay was conducted in the presence of IL-4 (days 0–4) plus IL-21 (days 2–4) to assess B cell differentiation by the plasma cell marker (CD138, Fas, GL7, and CD138). Alternatively, cognate and bystander B cells were distinguished by differentially labeling them with CFSE or CellTrace Violet dye.

In vitro T helper assay for dendritic cells

Bonemarrow-derived dendritic cells (BMDCs) were generated as previously described (22). Drug pretreated or untreated WT or Cd40lg-/- in vitro-generated Th1 cells were cocultured with BMDCs in the presence or absence of antigenic peptide, CsA (1 μM) or FK506 (0.1 μM), and isotype control Ab or anti-CD40L for 18 h. The level of CD70 on DCs was analyzed by flow cytometry. IL-6 production from DCs was analyzed by ELISA (eBioscience).

Statistical analysis

The p values were determined by the unpaired Student t test. All results are shown as the mean and the SD of the mean. A p value <0.05 was considered significant.

Results

Calcineurin inhibitors block de novo CD40L and cytokine synthesis but do not interfere with pCD40L surface mobilization

We proposed that pCD40L has a physiological role to selectively activate cognate APCs during brief encounters often observed in vivo. Because de novo CD40L and cytokines can cause general activation of APCs regardless of Ag specificity in vitro, we sought culture conditions in which de novo CD40L and other cytokines made by Th1 cells are blocked, but surface mobilization of pCD40L is preserved. Although we used cycloheximide to block de novo CD40L, and cytokines but to preserve pCD40L in the previous study (7), cycloheximide could not be used in the present study because it inhibited the upregulation of activation markers on APCs, as expected (data not shown). We also conducted experiments with the irreversible protein synthesis inhibitor emetine. However, we found that T cells pretreated with emetine and extensively washed released sufficient emetine to inhibit APC activation (data not shown). We then tested the calcineurin inhibitors CsA or FK506, known to inhibit de novo CD40L expression (23). CsA does not interfere with CD40-mediated B cell activation by enforced expression of pCD40L.
fixed, activated CD4+ T cells (24) or agonistic anti-CD40 (data not shown). These drugs also inhibit T cell production of IFN-γ and IL-4 (25). Upon stimulation with PMA plus ionomycin, Th1 cells showed evident pCD40L mobilization at 30 min (Fig. 1A, left) as well as robust induction and surface expression of de novo CD40L at 120 min (Fig. 1A, right). CsA or FK506 pretreatment completely inhibited activation-induced expression of de novo CD40L but had no effect on surface mobilization of pCD40L (Fig. 1B). The efficacy of CsA and FK506 was confirmed by complete suppression of IFN-γ production throughout this study, as shown in Fig. 1C.

pCD40L in Th1 cells selectively activates cognate B cells and promotes their proliferation and differentiation in vitro

To determine whether surface mobilization of pCD40L is sufficient for Ag-specific T cell help for B cells, we measured B cell activation. Cognate (antigenic peptide-pulsed) and bystander (unpulsed) B cells were differentially labeled with CFSE and were cultured with Th1 cells in the presence of CsA. Cd40lg−/− B cells were used as a negative control in separate cultures. CsA was maintained in the culture medium throughout the incubation period. CD40L-dependent upregulation of CD86, MHC class II, and ICAM-1 (CD54) and downregulation of CD62L were observed in cognate but not in bystander B cells after overnight culture (Fig. 2A). Increased size (forward scatter) and granularity (side scatter) were also observed in cognate B cells only (data not shown). By day 2, cognate but not bystander B cells upregulate IL-21R in a CD40-dependent manner (Fig. 2B). By these measures of B cell activation, the stimulatory capacity of pCD40L (with CsA) was comparable to that of de novo CD40L (no drug) (Supplemental Fig. 1). By these measures of B cell activation, the stimulatory capacity of pCD40L (with CsA) was comparable to that of de novo CD40L (no drug) (Supplemental Fig. 2) activation, the stimulatory capacity of pCD40L (with CsA) was comparable to that of de novo CD40L (no drug) (Supplemental Fig. 2A). By these measures of B cell activation, the stimulatory capacity of pCD40L (with CsA) was comparable to that of de novo CD40L (no drug) (Supplemental Fig. 2A).

Although we observed that pCD40L in Th1 cells generated in vitro can mediate cognate B cell activation, we were concerned that this finding could be an artifact of T cells activated in vitro. Therefore, we conducted a similar experiment using in vivo-generated effector CD4+ T cells. To generate effector CD4+ T cells in vivo, purified naive CD4+ T cells from DO11.10 Rag2−/− mice were transferred into nude mice, followed by s.c. immunization with papain plus OVA. Six days after immunization, cells from dLNs showed a robust expansion of effector CD4+ T cells as well as a vigorous GC reaction in an immunized mouse (Fig. 4A) compared with an unimmunized nude mouse (Fig. 4B), indicating that in vivo-generated effector CD4+ T cells are capable of helping B cells in vivo. The expanded CD4+ T cells are CD44hi effector cells and possess abundant pCD40L (Fig. 4C and data not shown). Highly purified (>95%) in vivo-generated effector CD4+ T cells were used in an ex vivo T helper assay in the presence of CsA. Similar to in vitro-generated Th1 cells, in vivo-generated effector CD4+ T cells are capable of activating cognate, but not bystander, B cell activation in a pCD40L-dependent manner as shown by increased ICAM-1 expression (Fig. 4D).
pCD40L can trigger DC activation

Another important function of CD40L is licensing of DCs, which is crucial for generating effective CD4+ and CD8+ T cell responses (4). Taking advantage of the fact that in vitro-generated Th1 cells do not produce detectable IL-6 (data not shown), we measured IL-6 production from BMDCs to evaluate the ability of pCD40L to activate DCs. WT or Cd40lg−/− DO11.10 Th1 cells were cocultured with BMDCs in the presence or absence of antigenic peptide and CsA or FK506 for 18 h. Although the magnitude of IL-6 production is significantly lower in the CsA- or FK506-treated groups than in the untreated group, treated Th1 cells still clearly induce IL-6 production from BMDCs in a peptide dose- and CD40L-dependent manner (Fig. 5A). CsA or FK506 treatment did not inhibit IL-6 production from DCs upon LPS stimulation (data not shown). To exclude potential differences between WT and Cd40lg−/− Th1 cells other than CD40L itself, we conducted similar experiments using WT in vitro-generated Th1 cells in the presence of either control Ab or neutralizing anti-CD40L. This experiment yielded a similar result (Fig. 5B). We also evaluated expression of CD70, which is known to be upregulated by a CD40 signaling and facilitates primary and secondary CD8+ T cell responses (26). We observed that Th1 cells induced upregulation of CD70 on DCs in the presence of antigenic peptide in a CD40L-dependent fashion in the presence of CsA or FK506 to levels comparable to the drug untreated group, suggesting that pCD40L is sufficient to efficiently induce CD70 upregulation on DCs (Fig. 5C). The efficacy of CsA and FK506 was confirmed by complete suppression of T cell IFN-γ production induced by DCs in the presence of antigenic peptide (Fig. 5D). Taken together, these results show that pCD40L can also mediate T cell-dependent DC activation.

The mobilization of pCD40L uses distinct machinery from that of lytic granules

Our previous study indicated that pCD40L exists in secretory lysosomes, a category of secretory vesicles that includes the lytic...
granules of CTLs and NK cells (7). To investigate the molecular mechanism of pCD40L mobilization from cytoplasm to cell surface, we used ashen mice, which are defective in release of lytic granule contents due to a mutation in the small GTPase Rab27a (18). First, we compared the surface mobilization of pCD40L with CD107a in polyclonal Th1 cells generated with CD4+ T cells from ash/+ or ash/ash mice. The mobilization of CD107a to the cell surface is an indication of the release of lytic granules (27). As expected, ash/ash Th1 cells have defective CD107a mobilization following all three stimuli tested (Fig. 6A). However, the mobilization of pCD40L is unimpaired in ash/ash Th1 cells (Fig. 6B). To confirm this phenotype using in vivo-generated effector CD4+ T cells, ashen mice were bred with SMARTA TCR transgenic mice. WT recipient mice of ash/+ or ash/ash SMARTA CD4+ T cells were infected with LCMV. On day 8 postinfection, splenocytes from infected animals were used ex vivo for evaluation of pCD40L mobilization (Fig. 7A, 7B). Whereas the mobilization of CD107a is clearly impaired in ash/ash SMARTA effector CD4+ T cells following antigenic stimulation (Fig. 7C), pCD40L mobilization is maintained (Fig. 7D). We conclude that effector CD4+ T cells use distinct machinery for the mobilization of pCD40L from that of lytic granules.

Discussion

In vivo studies using CD40L knockout mice and neutralizing anti-CD40L Ab have firmly established that CD40L mediates T cell help (2). In the classic model, effector CD4+ T cells deliver de novo CD40L, along with cytokines, to cognate APCs during prolonged interactions lasting hours (5). However, recent imaging studies with two-photon microscopy show clearly that effector CD4+ T cells do not usually have enough time to synthesize de novo CD40L. Given that effector CD4+ T cells also store intracellular pCD40L and mobilize it to the cell surface immediately after TCR stimulation, we proposed that pCD40L can mediate cognate APC activation during the brief Ag-specific interactions that dominate in vivo. In the present study, we blocked the synthesis of de novo CD40L and soluble cytokines while preserving the surface mobilization of pCD40L in effector CD4+ T cells, and demonstrated that pCD40L can selectively deliver T cell help to cognate B cells and trigger DC activation.

We speculate that effector CD4+ T cells deliver help to Ag-specific B cells using pCD40L at the T zone–B zone boundary, at extrafollicular sites, and in the GC (3). In situ staining studies have detected CD40L staining in all of locations listed above (6, 28). Studies with neutralizing anti-CD40L clearly show that CD40L is indispensable not only for the initiation but also for the maintenance of GCs, as well as the differentiation and affinity maturation of GC B cells (29, 30). However, we think that pCD40L plays a minor role during stable interactions between effector CD4+ T cells and cognate B cells owing to the opportunity for abundant delivery of de novo CD40L and cytokines. B cells undergoing stable interaction with T cells seem to be

FIGURE 6. Surface mobilization of pCD40L in Th1 cells is Rab27a-independent. In vitro-generated polyclonal Th1 cells from ash/+ and ash/ash CD4+ T cells were analyzed for mobilization of CD107a (A) and pCD40L (B) upon stimulation with PMA plus ionomycin, anti-CD3 plus anti-LFA-1, or anti-CD3 plus anti-ICOS for 30 min. Numbers in histograms indicate the percentage of stimulated cells that mobilize CD107a or CD40L. The mobilization assay was conducted in the absence of either CsA or FK506 treatment. Data are representative of three independent experiments (n = 2–3/group).
to de novo CD40L would promote plasma cell differentiation of GC B cells by repressing Bcl-6 via upregulation of IRF-4 expression (43).

We propose that pCD40L plays a major role in facilitating the affinity maturation process during brief interactions between low-affinity GC B cells and T cells in the light zone of GCs, as suggested previously (6). Compared to high-affinity B cells, low-affinity B cells preferentially seed GC reactions (31, 32). Increased peptide/MHC expression on GC B cells regardless of BCR affinity via DEC205-mediated Ag delivery enhanced plasma cell differentiation and compromised affinity maturation (44). This result may be explained partly by prolonged CD40 signaling owing to forced stable interactions between low-affinity GC B cells and T cells. Similarly, heightened CD40 signaling caused by agonistic anti-CD40 or a CD40L transgene in B cells also resulted in skewed short-lived plasma cell differentiation of GC B cells accompanied by premature termination of GC reactions, compromised affinity maturation, and diminished generation of long-lived plasma cells (45–47). A recent study suggested a model that explains how transient CD40 signaling facilitates affinity maturation of GC B cells by deletion of cells with damaged DNA (48). Brief CD40 signaling can induce temporary disruption of Bcl-6 repressor functions, promoting rapid, transient expression of DNA checkpoint genes and subsequent apoptosis of GC B cells with unrepaired DNA lesions (48). In fact, a fourth of T cells in GCs are associated with blebs from dead GC B cells (9). In this scenario, surviving GC B cells may undergo further affinity maturation by rapidly recovering Bcl-6 function (49). Whether a low-avidity T cell–B cell interaction is sufficient to induce the mobilization of pCD40L, as has been reported for preformed FasL (pFasL) in CTLs (50), needs to be addressed experimentally. A recent study showed that a transient BCR signaling increases B cell sensitivity to CD40L (51). This mechanism may also play an important role for optimizing a pCD40L-mediated selection process. Enrichment of both BCR and CD40 signaling signatures in the light zone compared with the dark zone of GCs by microarray analysis further supports this notion (44). Although our data showed that pCD40L could promote acquisition of plasma cell markers by cognate B cells, this may simply reflect favorable conditions for T cell help in vitro o wing to the lack of the GC environment that enforces intermittent CD40 signaling. Nevertheless, our data reveal the competence of pCD40L for B cell activation.

Another important function of pCD40L may be DC licensing. Two-photon microscopy shows predominantly brief interactions of effector memory CD4+ T cells with APCs in target tissues (11), suggesting a role for pCD40L during the effector phase of inflammation, probably through promoting cytokine secretion and upregulation of costimulatory molecules in DCs. Importantly, pCD40L has been implicated in the pathogenesis of rheumatoid arthritis (52). In this study, peripheral blood CD4+ T cells and synovial fluid CD4+ T cells from patients with rheumatoid arthritis, but not healthy donor CD4+ T cells, stored and mobilized pCD40L. Why healthy donor CD4+ T cells lacked pCD40L in this study is unknown, although it is possible that the sensitivity of the assay and/or human/mouse differences are contributing factors. The authors further showed, using stimulated and fixed patient CD4+ T cells, that the amount of pCD40L that came to the cell surface upon stimulation with PMA plus ionomycin was sufficient for CD40 signaling, as has been reported for preformed FasL (pFasL) in CTLs (50), needs to be addressed experimentally. A recent study showed that a transient BCR signaling increases B cell sensitivity to CD40L (51). This mechanism may also play an important role for optimizing a pCD40L-mediated selection process. Enrichment of both BCR and CD40 signaling signatures in the light zone compared with the dark zone of GCs by microarray analysis further supports this notion (44). Although our data showed that pCD40L could promote acquisition of plasma cell markers by cognate B cells, this may simply reflect favorable conditions for T cell help in vitro owing to the lack of the GC environment that enforces intermittent CD40 signaling. Nevertheless, our data reveal the competence of pCD40L for B cell activation.

Another important function of pCD40L may be DC licensing. Two-photon microscopy shows predominantly brief interactions of effector memory CD4+ T cells with APCs in target tissues (11), suggesting a role for pCD40L during the effector phase of inflammation, probably through promoting cytokine secretion and upregulation of costimulatory molecules in DCs. Importantly, pCD40L has been implicated in the pathogenesis of rheumatoid arthritis (52). In this study, peripheral blood CD4+ T cells and synovial fluid CD4+ T cells from patients with rheumatoid arthritis, but not healthy donor CD4+ T cells, stored and mobilized pCD40L. Why healthy donor CD4+ T cells lacked pCD40L in this study is unknown, although it is possible that the sensitivity of the assay and/or human/mouse differences are contributing factors. The authors further showed, using stimulated and fixed patient CD4+ T cells, that the amount of pCD40L that came to the cell surface upon stimulation with PMA plus ionomycin was sufficient to trigger IL-12 production from DCs. CsA-insensitive CD40L, presumably pCD40L, has been detected on CD4+ T cells from systemic lupus erythematosus patients (53, 54). These findings suggest a role for dysregulation of pCD40L in autoimmune settings. Possible regulatory functions of pCD40L [e.g., induction of...
IL-10 production from macrophages (55) or dampening innate immune responses by inhibiting inflammasomes (56) should also be included in considering pCD40L as a therapeutic target for autoimmune or inflammatory diseases (57). The mobilization of pCD40L is Rab27a-independent, indicating that pCD40L is stored in compartments distinct from lytic granules. Because the acquisition of lytic granules in CD4+ T cells is limited to Th1 effector cells (58), our recent finding of pCD40L expression in CD4+ single-positive thymocytes, Th2 cells, and resting memory CD4+ T cells provides further evidence that the pCD40L-containing compartment is distinct from lytic granules (7) (Y. Koguchi, A. Buenafe, T. Thauland, J. Gardell, E. Bivins-Smith, D. Jacoby, M. Sliıyla, and D. Parker, manuscript in preparation). Although pFaSL has been observed in lytic granules (59), recent reports indicate that pFaSL also resides in compartments distinct from lytic granules because surface expression of pFaSL is resistant to a microtubule inhibitor that blocks release of lytic granules (60) and occurs at a lower stimulation threshold than that of lytic granules (50). Our previous study showed that pCD40L is colocalized strongly with FaSL in Th1 cells (7). Taken together, these results suggest the existence of a distinct trafficking mechanism for rapid delivery of pCD40L and other TNF family members to the cell surface in CD4+ T cells. Evidence is accumulating that other TNF superfamily members are stored in secretory compartments in hematopoietic cells, including CD70 and CD40L in DCs (61, 62) and pCD40L and LIGHT in platelets (63, 64). Investigation of this trafficking machinery will provide methods to dissect the functions of preformed versus newly synthesized CD40L in vivo through the generation of knockout and transgenic mice lacking one or the other. It might also offer insight into the regulation of the many biological functions mediated by other TNF family members. Such efforts will provide new therapeutic targets for immunosuppression, including methods to further increase the efficacy of calcineurin inhibitors for established inflammatory conditions by blocking residual T cell-dependent APC activation triggered by pCD40L.

Acknowledgments

We thank Dr. Susan Murray for reviewing this manuscript. We thank Dr. Mark Sliıyla for providing LCMV stock, Dr. Jim Lindsay Whinton (The Scripps Research Institute) for providing SMARTA mice, Dr. Miguel Sea- bra (Imperial College London) for providing ashen mice on a C57BL/6 background, and Dr. David Hinrichs (Veterans Affairs Medical Center) for providing CD40-/- spleens. We also thank Katelynne Gardner Toren and Fanny Polleso for excellent technical assistance.

Disclosures

The authors have no financial conflicts of interest.

References

12. Casamayor-Palegra, M. Khan, I. C. MacKenzie, and J. Gardell, E. Bivins-Smith, D. Jacoby, M. Sliyla, and D. Parker, manuscript in preparation. Although pFaSL has been observed in lytic granules (59), recent reports indicate that pFaSL also resides in compartments distinct from lytic granules because surface expression of pFaSL is resistant to a microtubule inhibitor that blocks release of lytic granules (60) and occurs at a lower stimulation threshold than that of lytic granules (50). Our previous study showed that pCD40L is colocalized strongly with FaSL in Th1 cells (7). Taken together, these results suggest the existence of a distinct trafficking mechanism for rapid delivery of pCD40L and other TNF family members to the cell surface in CD4+ T cells. Evidence is accumulating that other TNF superfamily members are stored in secretory compartments in hematopoietic cells, including CD70 and CD40L in DCs (61, 62) and pCD40L and LIGHT in platelets (63, 64). Investigation of this trafficking machinery will provide methods to dissect the functions of preformed versus newly synthesized CD40L in vivo through the generation of knockout and transgenic mice lacking one or the other. It might also offer insight into the regulation of the many biological functions mediated by other TNF family members. Such efforts will provide new therapeutic targets for immunosuppression, including methods to further increase the efficacy of calcineurin inhibitors for established inflammatory conditions by blocking residual T cell-dependent APC activation triggered by pCD40L.

Acknowledgments

We thank Dr. Susan Murray for reviewing this manuscript. We thank Dr. Mark Sliyla for providing LCMV stock, Dr. Jim Lindsay Whinton (The Scripps Research Institute) for providing SMARTA mice, Dr. Miguel Seabra (Imperial College London) for providing ashen mice on a C57BL/6 background, and Dr. David Hinrichs (Veterans Affairs Medical Center) for providing CD40-/- spleens. We also thank Katelynne Gardner Toren and Fanny Polleso for excellent technical assistance.

Disclosures

The authors have no financial conflicts of interest.

References


He, J. S., D. E. Gong, and H. L. Ostergaard. 2010. Stored Fas ligand, a mediator of rapid CTL-mediated killing, has a lower threshold for response than degranulation or newly synthesized Fas ligand. *J. Immunol.* 184: 555–563.


