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Redox Remodeling by Dendritic Cells Protects Antigen-Specific T Cells against Oxidative Stress

Anna Martner,*† Johan Aurelius,*† Anna Rydström,*† Kristoffer Hellstrand,*† and Fredrik B. Thoren*†

Microorganisms and microbial products induce the release of reactive oxygen species (ROS) from monocytes and other myeloid cells, which may trigger dysfunction and apoptosis of adjacent lymphocytes. Therefore, T cell-mediated immunity is likely to comprise mechanisms of T cell protection against ROS-inflicted toxicity. The present study aimed to clarify the dynamics of reduced sulfhydryl groups (thiols) in human T cells after presentation of viral and bacterial Ags by dendritic cells (DCs) or B cells. DCs, but not B cells, efficiently triggered intra- and extracellular thiol expression in T cells with corresponding Ag specificity. After interaction with DCs, the Ag-specific T cells acquired the capacity to neutralize exogenous oxygen radicals and resisted ROS-induced apoptosis. Our results imply that DCs provide Ag-specific T cells with antioxidative thiols during Ag presentation, which suggests a novel aspect of DC/T cell cross-talk of relevance to the maintenance of specific immunity in inflamed or infected tissue. The Journal of Immunology, 2011, 187: 6243–6248.

Bacteria and viral products activate the NADPH oxidase of myeloid cells, which leads to the formation of reactive oxygen species (ROS; or “oxygen radicals”) (1–3). ROS are antimicrobial substances that kill ingested microbes, but they may also compromise the function and viability of adjacent cells when released into the extracellular space (4, 5). For example, T cells and other lymphocytes rapidly become inactivated and eventually undergo apoptosis when exposed to ROS (6–9). Because effector T cells commonly exert their functions in the oxidative environment characteristic of infected or inflamed tissues (10, 11), it is conceivable that T cells use strategies to escape oxygen radical-induced inactivation.

Earlier studies implied that molecules carrying reduced sulfhydryl groups (thiols), such as glutathione, thioredoxin, and cysteine, neutralize ROS and, thus, protect several types of cells from oxidative stress (12–14). The specific role of thiols for the function and survival of T cells in an environment of oxidative stress is not known. However, expression of thiols reportedly promotes T cell function; for example, the presence of reduced intracellular thiols (ic-thiols) in T cells and thiols expressed in cell surface proteins (cs-thiols) improves T cell reactivity and proliferation (15–17). The purported function of thiols in T cell immunity is further bolstered by the findings that dendritic cells (DCs), which are prominent APCs, release thiols (18, 19) and trigger thiol expression on adjacent cells, including NK cells and T cells (18, 20).

The present study sought to clarify the mechanisms and functional aspects of redox remodeling in T cells during Ag presentation, with a focus on interactions between DCs and Ag-specific T cells. Our findings provide a novel mechanism of DC/T cell cross-talk and point to a role for thiols in upholding the viability and function of effector T cells in an environment of oxidative stress.

Materials and Methods

Abs and reagents

The fluorochrome-labeled anti-human mAbs used were anti-CD4 (R-PE), anti-CD8 (R-PE/Ch7.14), anti-CD69 (FN 50), anti-CD71 (SHE 72), anti-CD3 (S4.1) (Invitrogen, Carlsbad, CA); and anti–CD86 (FUN-1), anti–HLA-DR (L243) (all from BD Biosciences, San Diego, CA); and anti-CD3 (S4.1) (Invitrogen, Carlsbad, CA); and anti–TLR-7 (E17.5F3.15.13), anti–TLR-8 (VJ22 (IMMU 546), and CMV Pp65/tra¬mer (HLA-A*0201/PE, peptide NLYPMATLV) (all from Beckman Coulter, Marseille, France). Purified anti–ICAM-1 (HA58) was from BD Biosciences, and purified anti–LFA-1 (7E4) was from Beckman Coulter.

The reagents used were Ficoll-Hypaque and Lymphoprep (Nycomed, Oslo, Norway); BSA (ICN Biomedicals, Aurora, OH); HRP (Boehringer-Mannheim, Mannheim, Germany); glutamate (BDH, Pool, Dorset, U.K.); EDTA, H2O2, isoluminol, maleimide, probenecid, monochlorobimane (MCB), 2-ME, LPS, staphylococcal enterotoxin B (SEB) (all from Sigma-Aldrich, St. Louis, MO); Alexa Fluor 633-conjugated C5-maleimide (ALM-633), CellTracker Violet, ProLong Gold antifade stain with DAPI, and LIVE/DEAD Fixable Violet Dead Cell stain (ViViD) (all from Invitrogen); NLVPMVATV peptide (Biopeptide, San Diego, CA); IL-4 and GM-CSF (R&D Systems, Abingdon, U.K.); and CyoMaxOx (PAA Laboratories, Pasching, Austria).

Cell isolation and DC generation

PBMCs were prepared from blood donor buffy coats, as described previously (3), and separated into CD14+ monocytes, CD3+ T cells, or CD19+ B cells using iMag positive selection beads (BD Biosciences), according to the manufacturer’s instructions. The isolation procedures resulted in >98% pure cell populations. T and B cells were immediately frozen in CyoMaxOx S. Purified monocytes were differentiated into DCs using IL-4 (500 U/ml) and GM-CSF (600 U/ml), as described (20).
Coculture of APCs and T cells

DCs or B cells were pulsed for 2 h with SEA or SEB (0.4 μg/ml) in 96-well round-bottom plates. SEA activates human T cells bearing Vβ16 or Vβ22 but not Vβ17 TCR regions, whereas SEB activates Vβ17, but not Vβ16 or Vβ22, T cells (21). Ag-pulsed DCs were washed twice in the plates with medium of ambient temperature, and autologous T cells were added at an APC/T ratio of 1:3. In some experiments, DCs were preincubated with 5 μg/ml anti-ICAM-1 for 40 min before T cells were added, diluting the Ab concentration to 2.5 μg/ml in the coculture. Alternatively, T cells were preincubated with 5 μg/ml anti-LFA-1 for 40 min before being added to the DCs. The Ab concentration in the cocultures was 0.83 μg/ml. In other experiments, 2 mM glutamate was added to DCs during Ag pulsing, as well as to the DC/T cell cocultures.

For assessment of T cell proliferation, CD3+ T cells were stained with CellTracker Violet, according to the manufacturer’s instructions, and analyzed by flow cytometry after 3–4 d of APC/T cell coculture. In some experiments, 100 μM 2-ME was added to the cocultures.

Staining for thiols

After overnight APC/T cell incubation, T cell expression of ic-thiols, cs-thiols, and CD69 was assessed. Cells were incubated with 40 μM MCB in complete medium containing 2.5 mM probenecid for 20 min at room temperature (RT), washed, stained with ALM-633 in NaCl for 15 min on ice, washed, and stained with surface Abs. To maintain MCB staining, all steps were performed in the presence of 2.5 mM probenecid. Data were acquired using FACScanto II or FACSAria instruments (BD Biosciences), both equipped with three laser lines (405, 488, and 633 nm), and analyzed with FACSDiva software version 6.1.2 (BD Biosciences). Mean fluorescence intensity (MFI) values for MCB stainings were normalized against an internal control of unstimulated T cells.

CMV tetramer staining

HLA-A2+ donors with >1% of CD8+ T cells showing specific binding of a PE-conjugated HLA-A2/NLVPVMATV tetramer (CMV tetramer) were identified from blood donor buffy coats. DCs were pulsed with the matrix metalloproteinase inhibitor (1 μg/ml), washed, and cultured with autologous T cells. After 18 h, the thiol expression by Ag-specific T cells was determined by flow cytometry.

Confocal microscopy

After coculture with SEA- or SEB-pulsed DCs, 10,000 ALM-633-stained Vβ17+, Vβ16+, or Vβ22+ T cells were sorted out by FACS onto microscope slides using a BD FACSAria. The purity of sorted cells was >99%. Cells were mounted in ProLong Gold antifade stain with DAPI, and confocal microscopy was used to examine the expression of thiols by Ag-specific and nonspecific T cells. Human peripheral blood T cells were incubated with medium of ambient temperature, and autologous T cells were added at an APC/T ratio of 1:3. In some experiments, DCs were preincubated with 5 μg/ml anti-ICAM-1 for 40 min before being added to the DCs. The Ab concentration in the cocultures was 0.83 μg/ml. In other experiments, 2 mM glutamate was added to DCs during Ag pulsing, as well as to the DC/T cell cocultures.

H2O2 consumption assay

After overnight stimulation with APCs, activated (CD69+) and nonactivated (CD69-) T cells were sorted to >99% purity using FACS, T cells were resuspended in Krebs-Ringer glucose buffer (500,000 cells/ml) and incubated with 100 μM H2O2 for 15 min at RT. The remaining H2O2 was detected, as described previously (22), in a Mithras LB 940 plate reader (Berthold Technologies, Bad Wildbad, Germany). The peak luminescence value was normalized against the value obtained using unstimulated T cells. In some experiments, T cells were incubated with 10 μM unconjugated maleimide in NaCl for 15 min on ice prior to the assay to neutralize cell surface thiols.

T cell apoptosis assay

Sorted populations of CD69+ and CD69+ T cells were resuspended in complete medium (250,000 cells/ml) in the presence of H2O2. After overnight incubation, cells were stained with ViViD, and the percentage of apoptotic cells was determined by flow cytometry.

Statistical analysis

The expressions of thiol and CD69 by T cell populations were compared using two-tailed paired or unpaired t tests or, for multiple populations, one-way ANOVA, followed by the Bonferroni multiple comparison test. Oxygen radical consumption and apoptosis data were analyzed by repeated-measures ANOVA, followed by the Bonferroni multiple comparison test (*p < 0.05, **p < 0.01, ***p < .001).

Results

DCs induce expression of thiols by Ag-specific T cells

In a first set of experiments, we aimed to clarify the role of DC/T cell interactions for the expression of thiols by Ag-specific and nonspecific T cells. Human peripheral blood T cells were incubated with autologous DCs presenting SEA or SEB, and the expression of cs- and ic-thiols was determined in the respective Ag-specific T cell subset. As shown in Fig. 1, DCs pulsed with SEA or SEB triggered a striking expression of cs-thiols by Ag-specific T cells (i.e., cells with TCR Vβ regions corresponding to the respective bacterial Ag). In addition to triggering cs-thiols, Ag-pulsed DCs induced ic-thiols in T cells with corresponding Ag specificity (Fig. 1A, 1C). Thus, DCs pulsed with SEA triggered cs- and ic-thiol expression by T cells with TCR Vβ16 and Vβ22 regions (specific to SEA), whereas SEB trigged thiol expression by Vβ17+ T cells (specific to SEB). The Ag-specific induction of thiols was observed in CD4+ and CD8+ T cell subsets (Fig. 1B, 1C) and was detectable after ~3 h of DC/T cell coculture, with sustained expression for several days (data not shown). Fig. 1D shows confocal micrographs of cs-thiol expression by Ag-specific and nonspecific T cells after interaction with DCs pulsed with SEA or SEB. Upon coculture with Ag-pulsed DCs, CD4+ or CD8+ T cells with TCR/Vβ regions corresponding to the stimulatory Ag proliferated vigorously, whereas T cells with noncorresponding TCR/Vβ regions did not proliferate (Supplemental Fig. 1A, 1B).

Induction of thiols in CMV-specific CD8+ T cells after interaction with DCs

The finding that DCs pulsed with bacterial Ags conveyed thiols to Ag-specific T cells incited us to examine whether the thiol induction in T cells could be reproduced by DCs presenting a viral Ag. DCs generated from HLA-A2+ blood donors with CMV tetramer+ CD8+ T cells were pulsed with a CMV peptide (NLVPVMATV) and cocultured with autologous T cells. The following day, the expression of cs-thiols and the activation marker CD69 by CD8+ T cells was determined by flow cytometry. CMV tetramer+ CD8+ T cells were specifically activated, as determined by CD69 expression. In accordance with the results using DCs pulsed with bacterial Ags, there was a pronounced induction of cs-thiols on CMV tetramer+ CD8+ T cells but not on nonspecific T cells (Fig. 2).

Thiols are selectively induced by Ag-pulsed DCs

We next compared the capacity of DCs and B cells to trigger Ag-specific thiol expression in T cells. DCs or B cells were pulsed with SEA or SEB and cocultured with autologous T cells. Although the pulsed DCs and B cells induced Ag-specific CD69 expression on T cells to a similar degree, DCs triggered significantly higher expression of cs-thiols and ic-thiols (Fig. 3) and proliferation (Supplemental Fig. 1C) in Ag-specific T cells. Similar results were obtained using CD4+ and CD8+ T cells (data not shown). In accordance with these findings, the bulk of SEA- or SEB-specific T cells (i.e., CD69+ cells) expressed significantly higher levels of cs- and ic-thiols when activated by Ag-pulsed DCs than after coculture with Ag-pulsed B cells (Supplemental Fig. 2).

T cells activated by DCs neutralize H2O2 and escape oxidant-induced apoptosis

After Ag presentation in lymphoid tissue, Ag-specific T cells home to the site of infection where they combat infection in a potentially hostile oxidative microenvironment (10, 11). To clarify the functional consequences of DC-induced thiol expression by T cells, we tested whether DC-stimulated T cells had acquired the capacity to neutralize oxygen radicals. T cells that had been incubated over-
night with SEB-pulsed DCs or similarly pulsed B cells were FACS sorted into CD69+ and CD69− populations and subsequently exposed to H2O2 as the source of ROS. As shown in Fig. 4, DC-stimulated CD69+ T cells, which expressed the highest levels of cs-thiols, were significantly more efficient consumers of H2O2 than was the corresponding CD69− subset or T cells stimulated by Ag-pulsed B cells. Neutralization of cs-thiols, which was achieved by the thiol-reactive reagent maleimide, efficiently reduced the oxygen radical consumption by thiol-expressing FACS-sorted CD69+ T cells (data not shown).

The finding that DC-stimulated T cells neutralized ROS prompted us to investigate whether increased thiol expression by DC-stimulated T cells conferred resistance to oxidant-induced apoptosis. Sorted CD69+ and CD69− T cell populations were exposed to H2O2 (100 or 200 μM) overnight, followed by assessment of T cell apoptosis. As shown in Fig. 5A and 5B, stimulation with Ag-pulsed DCs resulted in a specific protection of T cells with Ag specificity; hence, the CD69+ T cells activated by SEB-pulsed DCs were significantly less susceptible to oxidant-induced apoptosis than were non-SEB–specific CD69− T cells or unstimulated T cells. CD69+ T cells activated by SEB-pulsed B cells were not protected against oxygen radical-induced apoptosis compared with CD69− T cells or resting T cells (Fig. 5C).

Discussion

Effector T cells commonly exert their functions in tissues subjected to oxidative stress (10, 11), but the mechanisms explaining how Ag-specific T cells survive in these hostile environments remain incompletely understood. This study showed that DCs furnish effector T cells with cs- and ic-thiols during presentation of viral and bacterial Ags and that T cells with enhanced thiol expression consume ROS and escape oxidant-induced apoptosis. In accordance with previous studies (18, 20), a slight, but significant, increase in thiol expression was observed on all T cells cocultured with DCs. However, using Ag-pulsed DCs and detection of Ag-specific TCR/Vβ regions or tetramer technology, we found that the DC-induced thiol expression was several-fold higher in T cells specific for the presented Ag.

The conclusion that thiol induction and resistance to oxidant-induced apoptosis are more pronounced in, or even restricted to, the Ag-specific T cell subsets is illustrated by the experiments using SEA and SEB. DCs presenting SEA induced thiol expression in Vβ16+/Vβ22+ T cells but not in Vβ17+ T cells, whereas DCs or SEB-pulsed DCs (original magnification ×126). Arrows indicate Ag-specific T cells showing high thiol expression. *p < 0.05, **p < 0.001.
presenting SEB induced thiol expression in Vβ17+ T cells but not in Vβ16+/Vβ22+ T cells. Only the Ag-specific T cell subsets acquired resistance to oxidant-induced apoptosis. Following immunization there is a pronounced increase in free thiols in lymphoid tissue (23). These soluble thiols are assumed to be generated, at least in part, by DCs and other APCs that release extracellular thiols upon interaction with T cells (18, 19, 23, 24). Uptake of cystine by DCs via the cystine/glutamate antiporter with ensuing release of cysteine and other antioxidative thiols was suggested to contribute to a reducing milieu in the extracellular space (12, 18, 19). To clarify the putative role of the cystine/glutamate antiporter for the observed induction of thiols in Ag-specific T cells, we added glutamate at a concentration that efficiently reduced the cystine/glutamate exchange (19). A significant accumulation of cs- and ic-thiols also was observed in SEA- and SEB-specific T cells in the presence of glutamate (Supplemental Fig. 3A,3B), thus implying that transmembrane exchange of cystine/cysteine is not a major mechanism accounting for thiol induction in Ag-specific T cells. Interaction between ICAM-1/LFA-1 was shown to be important for firm DC/T cell adhesion (25). Treatment of DCs with Abs against ICAM-1, before and

**FIGURE 3.** DCs are more efficient than B cells in triggering thiol expression by Ag-specific T cells. CD3+ T cells were stimulated overnight by SEA- or SEB-pulsed DCs or B cells. A, Expression of cs-thiols and CD69 on SEA-responsive T cells (Vβ16+, green) and SEB-responsive T cells (Vβ17+, red). B, Cs-thiol expression for Vβ16+/Vβ22+ and Vβ17+ T cells (MFI; n = 13 for DCs, n = 11 for B cells). C, ic-thiol expression for Vβ16+/Vβ22+ and Vβ17+ T cells (MFI; n = 6 for DCs, n = 3 for B cells). D, CD69 expression for Vβ16+/Vβ22+ and Vβ17+ T cells (percentage of positive cells; n = 13 for DCs, n = 11 for B cells). *p < 0.05, **p < 0.01, ***p < 0.001.

**FIGURE 4.** T cells activated by DCs are efficient consumers of H2O2. T cells stimulated overnight with SEB-pulsed DCs or B cells were FACS sorted into CD69+ or CD69- populations and incubated with 100 μM H2O2 for 15 min. Remaining H2O2 was determined using chemiluminescence. A, Representative experiment. B, Data are the mean ± SEM of results obtained in six donors. **p < 0.01.

**FIGURE 5.** T cells activated by DCs are protected from H2O2-induced apoptosis. T cells stimulated overnight with SEB-pulsed DCs (A and B) or B cells (C) were FACS sorted into CD69+ or CD69- populations and exposed to H2O2 (100 or 200 μM) for 20 h. The percentage of T cells undergoing apoptosis was determined by flow cytometry after staining with ViViD. Results are the mean ± SEM (n = 8 for DCs, n = 7 for B cells). **p < 0.01.
during coculture with T cells, significantly reduced the induction of cs- and ic-thiol in Ag-specific T cells (Supplemental Fig. 3C, 3D). Hence, thiols provided during cell–cell contact in the DC-T cell synapse, as well as additional signals yet to be identified, are likely to account for the DC-induced thiol induction in Ag-specific T cells.

In our study, DCs were significantly more efficacious than B cells in triggering thiol expression in Ag-specific T cells; consequently, only DCs conferred T cell resistance to oxidative stress. Furthermore, although DCs and B cells triggered T cell activation (i.e., induction of cell surface CD69) to a similar extent, DCs induced significantly more Ag-specific T cell proliferation than did B cells (Supplemental Fig. 1C). Because thiol expression by T cells was reported to determine their proliferative capacity (16), it may be hypothesized that the advantage of DCs over B cells in triggering T cell thiol expression contributes to the enhanced T cell proliferation after stimulation by DCs compared with B cells. The mechanism explaining the greater capacity of DCs over B cells to induce T cell thiol expression remains to be established, but the finding that DCs express much higher levels of cs-thiols than B cells (Supplemental Fig. 4A) may be of relevance for this phenomenon. We observed that the addition of soluble thiols, in the form of 2-ME, to B/T cell cocultures only marginally increased T cell thiol expression and proliferation (data not shown), thus implying that the relative deficiency of B cells to induce thiol expression and proliferation in T cells is not merely the result of a reduced capacity to provide soluble thiols.

The expression of cs-thiols by DCs was only modestly affected by LPS stimulation. Also, LPS activation did not significantly enhance the capacity of DCs to induce thiol expression in Ag-specific T cells (Supplemental Fig. 4B). Because immature DCs were highly efficient in inducing thiol expression in T cells, it seems reasonable to assume that the capacity of DCs to induce thiol expression in T cells cannot be further enhanced by maturation. These data are in accordance with a previous study showing that immature and mature DCs confer a similar level of protection from oxygen radical-induced inactivation to cocultivated lymphocytes (20).

The mechanisms of T cell resistance to oxidant-induced inhibition and apoptosis may be of relevance for a broad spectrum of pathologies, including cancer, autoimmunity, and chronic infections. In cancer, ROS produced by myeloid-derived suppressor cells are assumed to inhibit cytotoxic T cell responses (26, 27) in contrast, a reduced ROS production in mice with an allelic polymorphism of cs- and ic-thiol in Ag-specific T cells (Supplemental Fig. 3C, 3D). Hence, thiols provided during cell–cell contact in the DC-T cell synapse, as well as additional signals yet to be identified, are likely to account for the DC-induced thiol induction in Ag-specific T cells.

In summary, our results implied that DCs equip T cells with antioxidant thiols during Ag presentation and that thiol induction is critical for the survival of T cells in an oxidative milieu. Thiol induction and resistance to oxidant-induced apoptosis are conveyed by DCs, but not by B cells, and restricted to the Ag-specific T cell subset. Our findings emphasize the importance of DC/T cell interactions for the evolvement of specific T cell-mediated immunity and provide a novel mechanism of T cell protection in inflamed or infected tissue.

Disclosures
The authors have no financial conflicts of interest.

References
Supplementary figure legends

SUPPLEMENTARY FIGURE 1. Antigen-pulsed DCs trigger antigen-specific T cell proliferation. A, Proliferation of Cell tracker Violet-stained SEA-specific (Vβ16+, green) or SEB-specific (Vβ17+, red) T cells after 3 d of stimulation with SEA- or SEB-pulsed DCs or non-pulsed control-DCs. In panel B and C the proliferative index is determined as percentage of T cells expressing Vβ16 or Vβ17 after 3-4 d of stimulation with SEA- or SEB-pulsed APCs, compared to the percentage of T cells expressing Vβ16 or Vβ17 in co-cultures with non-pulsed APCs. B, Proliferative index of CD3+, CD4+ and CD8+ Vβ16+ or Vβ17+ T cells after 3-4 d of stimulation with antigen-pulsed vs. non-pulsed DCs (N = 7, t-test). C, Proliferative index of Vβ16+ or Vβ17+ CD3+ T cells induced by antigen-pulsed vs. non-pulsed DCs and B cells (N = 6, paired t-test).

SUPPLEMENTARY FIGURE 2. CD69+ T cells activated by DCs express higher levels of thiols than non-activated or B cell-activated T cells. CD3+ T cells were stimulated overnight with SEA- (A-D) or SEB- (E-H) pulsed DCs or B cells and their expression of cs-thiols, ic-thiols and CD69 was determined by flow cytometry. A, E, Representative FACS-plots showing expression of cs-thiols and CD69 on stimulated T cells. B, F, Percentage of T cells expressing CD69 after stimulation with SEA or SEB. Expression of cs-thiols (MFI; Panel C and G) and ic-thiols (MFI; panel D and H) on gated CD69+ or CD69+ T cells. (For cs-thiols and % CD69+ cells N = 14 for SEA- and 16 for SEB-pulsed DC, N = 11 for SEA- and 13 for SEB-pulsed B cells. For ic-thiols N = 6 for pulsed DCs and N = 3 for pulsed B cells, One way ANOVA followed by Bonferroni’s multiple comparison test).
SUPPLEMENTARY FIGURE 3. Inhibition of ICAM-1 reduces the ability of DCs to trigger thiol expression in T cells. A-B, T cells were stimulated with SEA- or SEB-pulsed DCs in the presence or absence of 2 mM glutamate (glut). C-D, T cells were stimulated with SEB-pulsed DCs in the presence or absence of antibodies blocking LFA-1 (CD18) or ICAM-1 (CD54). The MFI of cs-thiols (A, C) and ic-thiols (B, D) by SEA responsive (Vβ16+/22+) or SEB responsive (Vβ17+) T cells are shown as percentage of control (data are mean ± SEM, N = 5 for glutamate and N = 3 for anti-LFA-1 and anti-ICAM-1, paired t-test).

SUPPLEMENTARY FIGURE 4. LPS-induced maturation of DCs does not alter their ability to trigger thiol expression by T cells. A, HLA DR, CD86 and cs-thiol expression by B cells, DCs and LPS-stimulated DCs. B, CD3+ T cells were stimulated with SEA- or SEB-pulsed DCs or similarly pulsed LPS stimulated DCs (LPS-DC). The following day the expression of cs-thiols and CD69 on SEA-responsive T cells (Vβ16+) and SEB-responsive T cells (Vβ17+) was determined using flow cytometry, (data are mean ± SEM, N = 4, One way ANOVA followed by Bonferroni’s multiple comparison test).