Corticosteroids enhance $CD8^+$ T cell–mediated airway hyperresponsiveness and allergic inflammation by upregulating leukotriene B_4 receptor 1

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Background: Leukotriene B_4 (LTB_4) is a potent inflammatory lipid mediator that binds to LTB_4 receptor 1 (BLT1). Ligation of BLT1 by LTB_4 plays an important role in the recruitment of effector memory $CD8^+$ T cells into the airways of sensitized and challenged mice.

Objectives: The effects of the corticosteroid dexamethasone (DEX) on BLT1-expressing effector memory $CD8^+$ T cells and effector memory $CD8^+$ T cell–mediated airway hyperresponsiveness (AHR) and allergic inflammation were determined.

Methods: Effector memory $CD8^+$ T cells were generated from $ovalbumin_{257-264}$–primed mononuclear cells from OT-1 mice in the presence of IL-2. In some cultures DEX was added. The effects of DEX on BLT1 expression, LTB_4-induced Ca^{2+} influx, phosphorylation of extracellular signal-regulated kinase 1/2, chemotaxis, and effector memory $CD8^+$ T cell–mediated AHR were examined.

Results: DEX-treated effector memory $CD8^+$ T cells showed significant increases in surface expression of BLT1, LTB_4-induced intracellular Ca^{2+} influx, phosphorylation of extracellular signal-regulated kinase 1/2, and chemotaxis. Upregulation of BLT1 by DEX was accompanied by increased IL-2 receptor expression. Adoptive transfer of DEX-treated effector memory $CD8^+$ T cells into ovalbumin-sensitized and ovalbumin-challenged $CD8^{-/-}$ mice resulted in significant increases in AHR, allergic inflammation, goblet cell metaplasia, and numbers of both $CD8^+$ and $CD4^+$ T cells in the bronchoalveolar lavage fluid and lungs.

Conclusions: Corticosteroids upregulate BLT1 on effector memory $CD8^+$ T cells and related signaling pathways and potentiate allergic airway inflammation and AHR induced by these cells. (J Allergy Clin Immunol 2008;121:864-71.)

Key words: *Central memory T cell, CD122, CD25, corticosteroid-insensitive asthma, effector memory T cell*

Abbreviations used

AHR:	Airway hyperresponsiveness
BAL:	Bronchoalveolar lavage
BLT1:	Leukotriene B_4 receptor 1
COPD:	Chronic obstructive pulmonary disease
DEX:	Dexamethasone
ERK:	Extracellular signal-regulated kinase
γc:	Common cytokine receptor γ chain
IL-2R:	IL-2 receptor
JNK:	Jun-N-terminal kinase
LTB_4:	Leukotriene B_4
MAPK:	Mitogen-activated protein kinase
OVA:	Ovalbumin
SIINFEKL:	$Ovalbumin_{257-264}$
T_{CM}:	Central memory $CD8^+$ T cell
T_{EFF}:	Effector memory $CD8^+$ T cell
WT:	Wild-type

Leukotriene B_4 (LTB_4) is an arachidonic acid–derived proinflammatory lipid mediator rapidly generated from activated innate immune cells, such as granulocytes, macrophages, and mast cells.[1] LTB_4 interacts with specific G protein–coupled receptors, LTB_4 receptor 1 (BLT1) and BLT2, and triggers a variety of leukocyte functions, including chemotaxis, degranulation, and the production of superoxide anion.[2,3] BLT1, the high-affinity receptor, is preferentially expressed on leukocytes, whereas BLT2, the lower-affinity receptor, is expressed more ubiquitously.[2,3] Ligation of BLT1 by LTB_4 is important for the activation and recruitment of inflammatory cells[4-12] to the sites of inflammation in various diseases,[13-16] including bronchial asthma.[17-22]

Two distinct populations of memory $CD8^+$ T cells have been defined based on their functional characteristics, migratory characteristics, or both.[23-26] One comprises the central memory $CD8^+$ T cells (T_{CM}s), which can be identified by the high expression of CCR7 and CD62L (L-selectin) and migrate preferentially to lymph nodes. The second, $CD62L^{lo}CCR7^{lo}CD8^+$ T cells, are labeled as T_{EFF}s that traffic more efficiently to nonlymphoid tissues and function more rapidly.[23-26] It is also known that BLT1 is expressed on T_{EFF}s but not on T_{CM}s.[11,12] We previously demonstrated that ligation of BLT1 on T_{EFF}s by LTB_4 was important for the recruitment of T_{EFF}s into the airways and contributed to the development of airway hyperresponsiveness (AHR) and allergic inflammation.[17,21]

Corticosteroids can effectively suppress inflammatory responses through repression of many immune genes by means of interaction with the glucocorticoid receptor and are widely used in the treatment of various inflammatory diseases. However, it has been shown that a proportion of asthmatic patients even had a

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decrease in lung function, despite high doses of inhaled or oral corticosteroid treatment.[27-29] Paradoxic effects of corticosteroids on neutrophils, such as increased LTB_4-induced intracellular Ca^{2+} mobilization and chemotaxis and enhanced survival, have been reported.[30,31] These effects are mediated by upregulating BLT1 expression.[30,31] Susceptibility to corticosteroids differs among T-cell subpopulations, states of maturity, or both.[32] Because administration of corticosteroids to asthmatic patients results in significant decreases in numbers of $CD4^+$ but not $CD8^+$ T cells in peripheral blood,[33] activated $CD8^+$ T cells are likely more resistant to corticosteroids than $CD4^+$ T cells. Therefore $CD8^+$ T_{EFF}s might play a more important role in the pathophysiology of inflammatory diseases than $CD4^+$ T cells after initiation of corticosteroid treatment.

In the present study we examined the effects of LTB_4 on intracellular signal transduction pathways and chemotaxis in T_{EFF}s and the effects of dexamethasone (DEX) on BLT1-expressing $CD8^+$ T_{EFF}s *in vitro* and T_{EFF}-mediated AHR and allergic inflammation *in vivo*. Furthermore, we defined the mechanism whereby DEX upregulates BLT1 expression on T_{EFF}s.

METHODS

Animals

Homozygous $CD8\alpha^{-/-}$ mice, OT-1 mice expressing a transgenic T-cell receptor specific for ovalbumin $(OVA)_{257\text{-}264}$ (SIINFEKL) peptide, and C57BL/6 wild-type (WT) mice were purchased from the Jackson Laboratory (Bar Harbor, Me). $BLT1^{-/-}$ mice were backcrossed into the C57BL/6 background for more than 9 generations.[6] BLT1-deficient OT-1 mice were generated by mating $BLT1^{-/-}$ mice with OT-1 mice. All mouse protocols used in this study were approved by the Institutional Animal Care and Use Committee of the National Jewish Medical and Research Center.

Generation of T_{EFF}s and T_{CM}s and DEX treatment

$CD8^+$ T_{EFF}s and T_{CM}s were differentiated *in vitro*, as previously described, with some modification.[12,34] Mononuclear cells from lymph nodes and spleens of OT-1 or BLT1-deficient OT-1 mice were stimulated with SIINFEKL (1 μg/mL) and cultured in complete medium containing 20 ng/mL of either recombinant murine IL-2 for the generation of T_{EFF}s or IL-15 (R&D Systems, Minneapolis, Minn) for T_{CM}s. To address the effect of DEX on memory $CD8^+$ T cells, DEX (Sigma-Aldrich, St Louis, Mo) was added to the culture during differentiation. Medium containing cytokine, DEX, or both was changed every day. In some experiments mononuclear cells or $CD8^+$ T cells selected negatively by means of magnetic cell sorting (Miltenyi Biotec, Auburn, Calif) from WT mice were stimulated with precoated anti-mouse CD3 mAb (2 μg/mL) and anti-mouse CD28 mAb (2 μg/mL, R&D Systems) for 2 days and then cultured the same way as SIINFEKL-primed $CD8^+$ T_{EFF}s. T_{EFF}s derived through these protocols showed phenotypic and functional characteristics of T_{EFF}s *in vivo*.[12,25,26] Cells were used between days 5 and 8 of culture after addition of cytokine.

Establishment of mouse anti-mouse BLT1 mAb

Mouse anti-mouse BLT1 mAbs were generated by immunizing the $BLT1^{-/-}$ mice with 300.19 cells expressing murine BLT1-RFP, as described elsewhere (Mathis S, et al, manuscript in preparation). Hybridoma culture supernatants of clone 3D7 were purified and fractionated over a Mono Q HR column (GE Healthcare, Buckinghamshire, United Kingdom). The peak fractions were biotinylated with the EZ-link sulfo-NHS-LC-biotin kit (Pierce, Rockford, Ill).

Flow cytometry

Biotinylated anti-mouse BLT1 mAb and allophycocyanin-streptavidin, fluorescein isothiocyanate–conjugated anti-CD122, and phycoerythrin-conjugated anti-CD25 (BD Pharmingen, San Jose, Calif) were used to detect surface expression of BLT1 and IL-2 receptor (IL-2R). Bronchoalveolar lavage (BAL) cells or lung mononuclear cells were stained with allophycocyanin-conjugated anti-CD3, fluorescein isothiocyanate–conjugated anti-CD4, and peridinin chlorophyll protein–conjugated anti-CD8 mAbs (BD Pharmingen) and analyzed by means of flow cytometry (FACSCalibur; BD Biosciences, San Jose, Calif) with CellQuest Pro software (BD Biosciences). The number of $CD4^+$ or $CD8^+$ T cells per lung was calculated by multiplying the percentage of stained cells by the total number of isolated lung mononuclear cells.

Intracellular Ca^{2+} mobilization

Cells (5×10^6 cells/mL) were loaded for 45 minutes at 37°C with 5 μM indo-1 acetoxymethyl ester (Invitrogen, Carlsbad, Calif) in RPMI-1640 medium containing 2% FCS. Cells were resuspended and subjected to analysis by means of flow cytometry (LSR; BD Biosciences) with FlowJo software (Tree Star, Ashland, Ore). After a baseline was established, cells were stimulated with LTB_4 (0-1000 nM, Sigma-Aldrich). Intracellular Ca^{2+} mobilization was determined by measurement of the median fluorescence ratio of indo-1 acetoxymethyl ester at 390 nm/490 nm emission.

Western blotting

Cells (4×10^6) were lysed with RIPA buffer containing protease inhibitors and phosphatase inhibitors (Sigma-Aldrich) on ice. Lysates were resolved by means of SDS-PAGE and transferred to nitrocellulose membranes. Blotted membranes were blocked in TBST buffer (25 mM Tris (pH 8.0), 125 mM NaCl, and 0.025% Tween-20) containing 5% nonfat dry milk and then incubated overnight at 4°C with TBST buffer containing 2% BSA with anti-phospho-extracellular signal-regulated kinase (ERK) 1/2, anti-phopho-p38 mitogen-activated protein kinase (MAPK), anti-phospho-Jun-N-terminal kinase (JNK; Cell Signaling Technology, Danvers, Mass), anti-ERK1, anti-p38 MAPK, or anti-JNK1 mAbs (Santa Cruz Biotechnology, Santa Cruz, Calif). Specific proteins were detected by using a chemiluminescence method with a horseradish peroxidase–conjugated anti-rabbit or anti-goat IgG antibody (Amersham Biosciences, Piscataway, NJ).

In vitro chemotaxis assay

LTB_4-induced chemotaxis was assessed by using 24-well transwell tissue-culture permeable supports with 3-μm pores (Corning, NY). LTB_4 solution (0-100 nM) was added to the lower wells, and cells were added to the upper chambers. After incubation for 1 hour at 37°C, the cells in the lower wells were counted.

Sensitization, adoptive transfer, and airway challenge

$CD8^{-/-}$ mice were sensitized intraperitoneally with 20 μg of OVA (Grade V; Sigma-Aldrich) emulsified in 2 mg of alum (Imject Alum, Pierce) on days 0 and 14. *In vitro*-generated T_{EFF}s or DEX-treated T_{EFF}s (5×10^6 cells/200 μL of PBS) were adoptively transferred intravenously through the tail vein into sensitized mice on day 28. Mice were challenged with OVA aerosols (1% in saline) for 20 minutes on days 28, 29, and 30 by using an ultrasonic nebulizer (OMRON, Kyoto, Japan). On day 31, airway responsiveness was measured as described below.

Assessment of airway function

Airway responsiveness was assessed as previously described by measuring changes in airway resistance and dynamic compliance in response to increasing doses of inhaled methacholine.[35] Data are expressed as the percentage of change from baseline values obtained after inhalation of saline.

BAL

Lungs were lavaged with 1 mL of Hank's balanced salt solution through the trachea immediately after assessment of AHR. Total leukocyte and differential

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