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Transcriptomic Analysis Reveals Receptor Subclass–Specific Immune Regulation of CD8+ T Cells by Opioids

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ABSTRACT

Opioid peptides are released at sites of injury, and their cognate G protein–coupled opioid receptors (OR) are expressed on immune cells. Exposure of human circulating CD8+ T cells to selective OR agonists differentially regulates thousands of genes. Gene set enrichment analysis reveals that μ-OR more strongly regulates cellular processes than δ-OR. In TCR naive T cells, triggering μ-OR exhibits stimulatory and inhibitory patterns, yet when administered prior to TCR cross-linking, a μ-OR agonist inhibits activation. μ-OR, but not δ-OR, signaling is linked to upregulation of lipid, cholesterol, and steroid hormone biosynthesis, suggesting lipid regulation is a mechanism for immune suppression. Lipid rafts are cholesterol-rich, liquid-ordered membrane domains that function as a nexus for the initiation of signal transduction from surface receptors, including TCR and μ-OR. We therefore propose that μ-OR–specific inhibition of TCR responses in human CD8+ T cells may be mediated through alterations in lipid metabolism and membrane structure. ImmunoHorizons, 2020, 4: 420–429.

INTRODUCTION

The CD8+ T cells are vital to maintain immune homeostasis and health by combating viral infections and recognizing tumor Ags while remaining nonresponsive to self-antigens (1–3). Immune cells respond to environmental and extracellular cues via surface receptors. Opioid receptors (ORs) are G protein–coupled receptors stimulated by endogenous opioid peptides and narcotic analgesics, which display immune regulatory activity (4, 5).

Endogenous opioid peptides are secreted at sites of injury by neurons, endocrine organs, and immune cells (6–13), and ORs are expressed on a wide variety of immune cells (14–16), leading to the proposal that endogenous opioids act as chemotactants and modulate immune function (4,17). Pharmacological opioids appropriate the endogenous opioid system, binding to and activating all three subtypes of OR: μ-, δ-, and κ-OR. Emerging literature suggests that certain classes of opioids interact with nonclassical ORs in the CNS, leading to complex physiological regulation (18). In the United States, pharmacological and street
opoid use has reached epidemic proportions (19). There are conflicting reports on the net outcome of opioid exposure to the adaptive immune system (5), but the overall profile seems to be immunoinhibitory, limiting inflammation and autoimmunity. In T cell–specific models, opioid administration prevents porcine CD8+ T cell–mediated delayed-type hypersensitivity reactions (20) and improves disease scores in multiple sclerosis patients (21, 22). Chronic opioid use in humans is associated with an imbalance in CD8+ T cell subsets, notably decreased T effector memory CD45RA+ cells and perturbed responses to opioid agonists and TCR stimulation ex vivo compared with cells from healthy controls (16). The kinetics observed indicate that an 18-h pre-exposure of CD8+ T cells to opioids is required to perturb TCR activation, indicating their mechanism of action is not to directly modulate the TCR signaling cascade (16). Therefore, we hypothesize that the distinct CD8+ T cell subset profile in response to in vivo and ex vivo opioid exposure is partially under transcriptional control.

Whereas genes giving rise to protein coding transcripts account for a minor subset of the human genome, noncoding RNA (ncRNA) genes, which are templates to transcripts that carry out their effector functions as RNA molecules, account for the vast majority of transcribed genomic sequences (23). Several housekeeping ncRNAs, such as transfer, ribosomal, and small nucleolar RNAs, have been extensively studied (24). The less well-characterized class of long ncRNAs (lncRNAs) are nonhousekeeping ncRNAs that range in length from ~200 to tens of thousands of nucleotides and function as regulatory molecules (25). lncRNAs are often transcribed in a cell type–specific manner, and their expression and function vary both with cell type and cell state (25). For example, lncRNAs expressed in immune cells can have lineage-specific activator and repressor functions (26–28). Of particular interest, long intergenic ncRNAs (lincRNAs) are a major subclass of lncRNAs that do not overlap with other annotated genes and, in several cases, have been shown to act on a variety of targets, thus contributing to the coordination of cellular processes (29).

Large-scale transcriptomic fluctuations are associated with T cell differentiation. After vaccination, virus-specific memory and effector human CD8+ T cells differ in protein coding and ncRNA profiles compared with each other and compared with naive cells (30). In addition, this RNA profile is further modulated with increasing time since vaccination, replicating murine models (30). During viral infection, both viral and host RNAs change in expression level (28, 31, 32), and correlations to clinical outcomes are emerging. For example, HIV elite controllers bear a different CD8+ T cell transcriptional signature than people with progressing HIV (33).

Although to date no transcriptomic studies on immune responses of CD8+ T cells exposed to opioids have been reported, high-throughput analysis in the CNS and mixed immune cell populations indicates that chronic opioid users have atypical protein coding and ncRNA transcriptional profiles (34–36). In this study, we investigate the early and cell type–specific transcriptomic regulation of CD8+ T cells by opioids and, to our knowledge, provide a first glimpse into the opioid-mediated control of key immune-related pathways, including lipid metabolism.

**MATERIALS AND METHODS**

### Human subjects

Participants were recruited in compliance with an approved Institutional Review Board protocol from the University Hospitals Cleveland Medical Center. Signed informed consent was obtained after explanation of the study and annually thereafter. Exclusion criteria included self-reported autoimmune disease, inflammatory bowel disease, chronic viral infection, and opioid use within the last 5 y. At the time of enrollment, participants completed an optional survey for self-reported demographic data, targeted medical history, and opioid use. Up to 120 ml of blood was collected in sodium heparin tubes. All donors reported no opioid use. The characteristics of the five donors are provided in Fig. 1A.

### Primary cell culture

PBMCs were isolated using a density gradient media (Lymphoprep; STEMCELL Technologies, Vancouver, Canada) and SepMate centrifugation tubes (STEMCELL Technologies), following the manufacturer’s instructions. Immediately after PBMC isolation, CD8+ T cells were isolated via negative selection (EasySep Human CD8+ T Cell Isolation Kit; STEMCELL Technologies). CD8+ T cells were washed twice with PBS and cultured in RPMI 1640 media (Corning, Corning, NY) supplemented with 10% screened FCS (Corning) and 5% HEPES (Genesee Scientific, El Cajon, CA) in 96-well U-bottom plates at 1 million cells/ml. Ten wells were treated for each condition (10 × 250 μl each, 2.5 million cells total) and pooled for RNA isolation.

### Ex vivo opioid and T cell activator treatment

The δ-OR selective agonist DPDEP [Ki (nanomolar) μ >1000, δ 14, κ >1000 (37, 38); Abcam, Cambridge, MA] or the μ-OR selective agonist morphine sulfate [Ki (nanomolar) μ 14, δ >1000, κ 538 (37, 38); Sigma-Aldrich, St. Louis, MO and Spectrum Chemical, New Brunswick, NJ] were dissolved in RPMI 1640 and added at 1 and 10 μM, respectively, for 18 h. ImmunoCult Human CD3/CD28 T Cell Activator (STEMCELL Technologies) was subsequently added for 6 h at a concentration specified by the manufacturer. Matching volumes of RPMI were added to untreated samples (experimental design, Fig. 1A).

### RNA extraction and sequencing library preparation

For donors 72171, 311552, and 271865, total cellular RNA was isolated using a PureLink Kit (Invitrogen, Carlsbad, CA). Genomic DNA was eliminated with TURBO DNA-free Kit (Invitrogen). Samples were analyzed using a bioanalyzer, and sequencing libraries were prepared with SMART-Seq v4 Ultra Low Input Kit for Sequencing (Takara Bio, Mountain View, CA).

For donors 201920 and 205007, cell pellets were flash frozen, and subsequently, total cellular RNA was isolated using an RNeasy Kit (QIAGEN, Germantown, MD). Purity was assessed using a bioanalyzer, and sequencing libraries were prepared with SMARTer Stranded Total RNA sequencing (RNA-seq) Kit V2 Pico Input Kit (Takara Bio).
RNA-seq analysis

Library preparation was followed by paired end sequencing using Illumina technology. Donor 721171, 311552, and 271865 samples were sequenced using a NextSeq 550 (75 bp, 66 million average reads per sample), and donors 201920 and 205007 were sequenced using a NovaSeq 6000 (150 bp, 80 million average reads per sample). In addition to the use of different reagents during the preparation steps, the two groups of samples were processed and sequenced several months apart to ensure reproducibility.

Sequencing alignment and quantification

After quality control and preprocessing with FastQC and Trim Galore, reads were aligned and quantitated to hg38 with GENCODE release 28 as the reference annotation using Kallisto/Sleuth for transcript-level expression analysis and STAR/HTseq for gene level analysis, which yielded concordant results. Identical biotypes for the same gene were merged. Differential expression was performed using edgeR and confirmed by per donor calculation of change in expression after library normalization.

Pathway analysis

Pathway analysis was performed on preranked lists of differentially expressed protein coding genes using all libraries of the Molecular Signatures Database (MSigDB) (v 7.0) database and the Gene Set Enrichment Analysis (GSEA) tool v 4.0.3 (39, 40). Gene set overlaps were computed using the Investigate Gene Sets tool from the University of California San Diego and Broad Institute GSEA Web site. Mapping to Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways was performed using the KEGG Mapper—Search and Color Pathway developed by Kanehisa Laboratories.
**Genome-wide association studies**

Genome-wide association studies (GWAS) catalog data files were downloaded from the National Human Genome Research Institute–European Bioinformatics Institute catalog of published GWAS (41). The number of genes in this dataset related to each trait was compared using Ensembl identifiers (IDs), treating all isoforms of a gene as one. Multiple single nucleotide polymorphism sites in a gene were counted separately.

**Data deposition**

RNA-seq data have been deposited under the Sequence Read Archive BioProject ID PRJNA637214.

**RESULTS**

Human blood-derived CD8⁺ T cells from opioid naive donors were treated with δ- or µ-OR selective agonists for 18 h, followed by the presence or absence of TCR cross-linking for 6 h, as described in the Materials and Methods and Fig. 1A, generating six treatment combinations for gene expression comparisons. The outcome of each OR on protein coding and lincRNA transcription in resting, TCR naïve CD8⁺ T cells was analyzed (Figs. 1, 2). The δ-OR–treated cells differentially expressed 650 protein coding genes, whereas the µ-OR–ligated cells differentially expressed 1608 genes compared with unstimulated cells. Analysis of the corresponding MSigDB hallmark pathways showed that µ-OR produced dramatically more positively enriched pathways than δ-OR when compared with unstimulated cells (Fig. 1B). The expression pattern of the 90 genes regulated by both receptors was tracked by individual donors (Fig. 1C), and the array of responses of these genes to receptor ligation was quantified (Fig. 1D), with 73% of the genes upregulated and 14% downregulated by both receptors. The identities and donor-specific expression of the 11 genes inversely regulated by δ-OR and µ-OR are shown in Fig. 1E. lincRNAs are also differentially regulated by the two receptors (Fig. 2A, 2B). In contrast to protein coding genes, a larger proportion of lincRNAs are downregulated by both ORs (19 out of 46). Eighteen of the differentially expressed lincRNAs (39%) were inversely regulated by the two ORs (Fig. 2C). Very few lincRNAs are cataloged in the

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**FIGURE 2.** In TCR naïve CD8⁺ T cells, lincRNA genes are regulated in an OR subtype-specific manner.

- **A** Heat map of lincRNA genes that show differential expression in all three donors in both δ-OR and µ-OR agonist treatment groups.
- **B** Quantification of lincRNA gene expression patterns in response to either the δ-OR or µ-OR agonists.
- **C** Expression patterns of the lincRNA genes inversely regulated by δ-OR (left) and µ-OR (right).
- **D** Top 15 GWAS traits associated with the protein coding and lincRNAs regulated by δ-OR (left) or µ-OR (right).
- **E** Quantification of protein coding and lincRNAs that have polymorphisms associated with immune or lipid-related traits via GWAS.

Donor ID is indicated to the right (A) or below (C) each heat map.

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MSigDB commonly used for pathway analysis, limiting the ability to predict their impact on cellular function. To circumvent this, we compared our data to GWAS, which can be used to predict diseases associated with coding and ncRNAs. The top 15 traits associated with genes regulated by each OR are shown in Fig. 2D, and select immunity and lipid-related traits and diseases are highlighted in Fig. 2E.

Stimulation of the TCR in CD8+ human T cells positively enriched several immune associated pathways, with a notable lack of negative enrichment apart from that of protein secretion (Fig. 3A). Thousands of individual protein coding genes were consistently up- or downregulated across all donors, with more than double the number of upregulated genes (1674) compared with downregulated (808) (Fig. 3B, 3C). Conversely, for lincRNAs, TCR cross-linking preferentially downregulated more genes (134) than it upregulated (55) (Fig. 3D, 3E).

To evaluate the effects of opioids on the subsequent immune activation via the TCR, the transcriptional profiles of cells from the same donor treated with a δ-OR or μ-OR agonist prior to TCR cross-linking were compared with those of opioid naive cells that were TCR cross-linked. The resulting gene expression pattern of δ-OR and μ-OR agonist pretreated cells showed changes in 1722 and 2157 genes, respectively. Pathway analysis indicated that compared with cells not pretreated with OR agonists, δ-OR and μ-OR pretreated and TCR cross-linked cells showed extensive downregulation of several cellular networks. Consistent with earlier findings, μ-OR once again had a more pronounced effect (Fig. 4A). The protein coding genes that were differentially expressed after pretreatment with either OR agonist prior to TCR cross-linking were predominantly downregulated (Fig. 4B, 4C), with 12% (46 of 398) inversely regulated by the two receptors (Fig. 4C). The identities and donor-specific regulation of these inversely regulated genes are shown in Fig. 4D. The majority of lincRNAs that are regulated by both ORs in the context of TCR cross-linking show increased expression (53%, or 39 out of 73) compared with opioid naive cells that are TCR cross-linked (Fig. 5A, 5B). Fourteen lincRNA genes are inversely regulated (Fig. 5B, 5C). The top 15 GWAS traits associated with genes regulated by pretreatment with an agonist for each OR are shown in Fig. 5D, and select immunity and lipid-related traits and diseases are presented in Fig. 5E.

We selected 131 genes involved in lipid metabolism that were either induced or inhibited by μ-OR ligation of TCR naive cells (Fig. 1), based on their involvement in cholesterol homeostasis, androgen response, fatty acid metabolism, estrogen response (early and late), and adipogenesis using the hallmark gene sets of MSigDB. We also included genes under the GWAS terms: total cholesterol levels, low-density lipoprotein cholesterol levels, and high-density lipoprotein cholesterol levels. To gain insight into how these processes are regulated by opioid treatment and TCR stimulation, we mapped the differentially expressed genes across treatment conditions to the Gene Ontology (GO) pathways with a false discovery rate cutoff of 0.05 (Fig. 6A). The expression patterns of these genes differ across treatment conditions and between the two receptors (Fig. 6B), with μ-OR triggering a stronger induction or inhibition of lipid gene expression than that of δ-OR. Genes for which OR ligation and TCR cross-linking have opposing effects but in which OR signaling predominates are shown in Fig. 6C. Comparing the genes regulated by μ-OR in CD8+ T cells to the KEGG pathway database, we propose a model in which opioid regulation of CD8+ T cell lipid homeostasis may modulate the assembly of lipid rafts (Fig. 6D).

**DISCUSSION**

**ORs enrich immunity-associated pathways in TCR naive CD8+ T cells**

The transcriptional outcomes of engaging the two ORs on CD8+ T cells, in the absence of TCR activation, dramatically differ, as demonstrated in Fig. 1B–E, with δ-OR regulating fewer protein coding genes (650) than μ-OR (1608) (Fig. 1B). The physiological consequence of this transcriptional difference between δ-OR and μ-OR is that δ-OR regulates a limited number of biological
pathways at a lower level, whereas \( \mu \)-OR regulates several processes associated with immune function (Fig. 1B). Notably, the transcriptional signature of \( \mu \)-OR agonist-treated CD8\(^+\) T cells reveals enhanced activity of the immune regulatory pathways: mTORC1, TNF-\( \alpha \)/NF-\( \kappa B \) pathway, IL-2/IL-15 signaling, and a general inflammatory response, suggesting an immune stimulatory function for \( \mu \)-OR. Ironically, triggering the \( \mu \)-OR simultaneously downregulated other immune pathways (IFN-\( \alpha \) and IFN-\( \gamma \)) that are strongly associated with CD8\(^+\) T cell function. These results underscore the intricacy of opioid regulation on immunity and help to emphasize that classifying an opioid as solely immunostimulatory or immunosuppressive is unwarranted (4). The complexity of opioid-mediated immune regulation is consistent with protein expression analyses that show inhibition of the immune activation markers, CD69 and CD25, by \( \mu \)-OR agonists was observed in only a small subset of CD8\(^+\) T cells (16).

Analyzing the protein coding genes that are regulated by both ORs reveals that the magnitude of opioid-induced gene regulation is donor specific, even for genes that are consistently up- or downregulated by either receptor (Fig. 1C). These data are supported by previous findings that OR expression on human CD8\(^+\) T cells shows broad interindividual variability (16). For the coding genes that are shared between the two receptors, the majority (66 genes) were upregulated by both receptors, whereas smaller numbers (13 genes) were downregulated by both (Fig. 1D).

Under scoring the OR-subtype specificity of this system, 11 genes showed discordant regulation by the two receptors (Fig. 1D, 1E). Although the 11 discordant genes do not map to a particular biological pathway (data not shown), they may be critical components of the opposing biological pathway responses described in Fig. 1B. Of note, OR gene expression was modified by OR ligation; \( \mu \)-OR ligation downregulated the expression of both \( \mu \)-OR and \( \delta \)-OR genes but not...
that of κ-OR, whereas δ-OR ligation downregulated only μ-OR (data not shown). This highlights the complexity of OR autoregulation as CD8\(^+\) T cells were shown not to regulate OR protein expression in response to 18 h of receptor ligation (16), yet at 24 h, OR gene expression is modulated by opioids (data not shown).

Regulatory lincRNAs are further points of divergence between the two receptors, and, similar to protein coding genes, lincRNAs common to both receptors may have the same (28 genes) or opposite (18 genes) response patterns (Fig. 2A–C). Similar to the GSEA analysis of protein coding genes, δ-OR has weaker associations than μ-OR when compared with GWAS studies (Fig. 2D).

### ORs regulate fatty acid metabolism in TCR naive CD8\(^+\) T cells

A notable finding is the upregulation of cholesterol homeostasis, fatty acid metabolic, bioacid metabolic, and steroid hormone pathways by μ-OR in the pathway analysis results and cholesterol and lipoprotein networks by both ORs in the GWAS analysis (Figs. 1B, 2E). Elevated cholesterol levels are associated with enhanced analgesia with opioid treatment, which is consistent with the observation that cholesterol enhances OR signaling by organizing them into lipid raft domains and potentially altering their downstream second messengers (42, 43). Lipid rafts are liquid-ordered, cholesterol-rich domains of the plasma membrane that function as a nexus for initiating receptor-mediated cell signaling (43–46), most notably in human T cells (46). The enhancement of cholesterol pathways in human CD8\(^+\) T cells by opioid exposure suggests that by regulating cholesterol metabolism, opioids may not only enhance their own signaling, they also modulate signal transduction from other lipid raft-associated receptors, including the TCR (46). Chronic opioid users have decreased serum triglyceride and cholesterol levels (36), suggesting that the cholesterol being synthesized may be integrated into

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**FIGURE 5.** The expression of lincRNAs regulated by TCR are influenced by previous OR ligation ex vivo. (A) Heat map of lincRNA genes that show differential expression in all three donors in both δ-OR and μ-OR agonist treatment groups. (B) Quantification of lincRNA gene expression patterns in response to either δ-OR or μ-OR pretreatment. (C) Expression patterns of the lincRNA genes inversely regulated by δ-OR (top) and μ-OR (bottom). Donor ID is indicated to the right (A) or left (C) of each row. (D) Top 15 GWAS traits associated with the protein coding and lincRNAs regulated by δ-OR (left) or μ-OR (right) in TCR-stimulated cells. (E) Quantification of protein coding and lincRNAs that have polymorphisms associated with immune or lipid-related traits via GWAS.
the membrane rather than transported. Our results extend previous studies (42, 43) by showing that opioids directly regulate the metabolic processes that contribute to their signal propagation.

CD8+ T cells undergo immediate transcriptomic changes in response to TCR stimulation

Although there are reports on the differing transcriptomic profiles of naive and memory CD8+ T cells weeks after immunization (30), the literature lacks a description of immediate changes because of TCR cross-linking, which informs on the mechanisms for CD8+ T cell activation. The transcriptomic response to TCR cross-linking in CD8+ T cells (Fig. 3A) matches the nuclear localization of inflammatory transcription factors in response to the TCR signaling cascade, largely studied in CD4+ T cells, resulting in upregulation of TNF-α, IFN-γ, IFN-α, and various cytokine responses (47). The immune pathways activated by TCR cross-linking both overlap and counter the pathways regulated by opioids, as seen in Figs. 1B and 2A. Of the thousands of protein coding genes regulated by the TCR (Fig. 3B, 3C), two out of three are upregulated. In contrast, far fewer lincRNAs are differentially expressed 6 h after TCR cross-linking, and 71% of those genes are downregulated (Fig. 3D, 3E). We propose that some of these lincRNA genes play a repressive role in T cell activation, and hence, inhibition of their expression may help in inducing the transcriptomic changes observed during T cell activation.

Opioid pretreatment ex vivo dampens a subsequent TCR response

Given the immunomodulatory effects of opioids on TCR naive cells (Fig. 1), we hypothesized that opioid pretreatment of TCR naive CD8+ T cells disrupts the response to the subsequent TCR cross-linking. Compared with opioid naive CD8+ T cells that were TCR cross-linked, T cells pretreated either with δ-OR or μ-OR agonists before TCR cross-linking showed differential regulation of thousands of genes (1722 genes and 2157 genes, respectively), inhibiting several immune pathways (Fig. 4A). Consistent with the results of opioid exposure of TCR naive CD8+ T cells shown in Fig. 1, μ-OR is a stronger immune modulator than δ-OR (Fig. 4A). In contrast to the effects of opioids on resting cells (Fig. 1), the effects of opioid pretreatment were largely inhibitory on protein coding genes (Fig. 4). Of the protein coding genes regulated by both ORs, the majority are downregulated (277 genes); far fewer are enriched by opioid pretreatment (75 genes) (Fig. 4B, 4C), which contrasts with the stimulatory effects of opioids in the absence of TCR (Fig. 1). Of 46 protein coding genes divergently regulated by the two receptors (Fig. 4C, 4D), five were associated with GO stress responses, apoptotic signaling due to endoplasmic reticulum stress (CEBPB, DDIT3, TRIB3, and BBC3), and transcription from the polymerase II promoter (CEBPB, DDIT3, and KLF2), all of which are upregulated by μ-OR, suggesting the stress response may differ between the two receptors. Again, in contrast to the coding genome, lincRNAs regulated by both receptors are predominantly

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overexpressed after TCR cross-linking in opioid pretreated cells compared with opioid naïve cells (Fig. 5A, 5B), and 14 were inversely regulated by the two receptors (Fig. 5B, 5C). A GWAS trait analysis revealed that cells with prior stimulation of either OR maintained differential expression of hundreds of genes associated with disease in humans (Fig. 5D) and with immunity and lipid metabolism (Fig. 5E). As was observed in TCR naïve T cells, ligating either OR regulates lipid metabolism, which is not masked by a subsequent, strong TCR stimulus (δ-OR, Fig. 5E; μ-OR, Figs. 4A, 5D, 5E). Taken together, these results re-emphasize the potential for opioids to regulate signal transduction from a wide variety of receptors that nucleate their response in the context of lipid rafts (43–46), including the TCR (46).

Lipid metabolism as a node of CD8⁺ T cell regulation

It is striking that multiple genes regulated by OR in CD8⁺ T cells are associated with lipid metabolism pathways. In addition, the expression patterns of those genes differs between the two receptors (Fig. 6B), with μ-OR triggering a stronger induction or inhibition of lipid gene expression than that of δ-OR. Expression of genes that are coordinately regulated by the two ORs and whose expression is opposingly regulated by TCR signaling in the absence of OR ligation is not reversed when T cells are first exposed to OR pretreatment and then stimulated by TCR cross-linking (Fig. 6C). Comparing the OR-regulated genes to the KEGG pathways database, we developed a model that may predict μ-OR regulation of lipid metabolism (Fig. 6D). In this model, inhibition of ACADVL and ACOX1 gene expression by μ-OR promotes anabolism of fatty acid precursors into long chain fatty acids and cholesterol, mediated by μ-OR transcriptional upregulation of such genes as HSD17B4, FADS2, SCD, and ELOVL6, enabling their assembly into lipid rafts. Transcriptional data alone does not encompass the complexity of cell physiology, and the model will need additional details provided by complementary in vitro assays on CD8⁺ T cells.

Overall, transcriptional data provide evidence that opioid treatment of TCR naïve CD8⁺ T cells yields a mixed immunostimulatory and immunoinhibitory response that is OR subtype specific. In contrast, opioid exposure in T cells that are subsequently activated by TCR cross-linking prevents upregulation of crucial immune pathways. We propose that one mechanism for this impaired immune activation may be lipid regulation, which may impact signal transduction from a wide variety of T cell surface receptors embedded in lipid rafts.

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DISCLOSURES

The authors have no financial conflicts of interest.

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