AWARD NUMBER: W81XWH-19-1-0375

TITLE: Neutrophil Elastase Reprograms Macrophage Function in Chronic Obstructive Pulmonary Disease

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CONTRACTING ORGANIZATION: Virginia Commonwealth University Children's Hospital of Richmond at VCU

REPORT DATE: OCTOBER 2022

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

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REPORT DOCUMENTATION PAGE

Form Approved
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1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
OCTOBER 2022	Annual	9/01/2021-8/31/2022

4. TITLE AND SUBTITLE

Neutrophil Elastase Reprograms Macrophage Function in Chronic Obstructive Pulmonary Disease

5a. CONTRACT NUMBER
W81XWH-19-1-0375

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Neutrophil elastase (NE) impairs phagocytic function. NE is taken up by human blood monocyte derived macrophages (hBMDM) and retains proteolytic activity in the macrophages, leading to cleaving of multiple targets. We discovered that NE clips Histone H3 in hBMDM resulting in chromatin decondensation and release of Macrophage Extracellular Traps (METs). We also discovered that NE cleaves histone deacetylases and sirtuin 1 resulting in unopposed acetyltransferase activity that causes translocation of a major alarmin, High Mobility Group Box 1 (HMGB1) from the nucleus to the cytosol. We have published three papers related to this project and are presenting two posters about this work this year. We will use an approved no cost extension to complete two proteomic analyses: 1) hBMDM cell lysates from control subjects and subjects with COPD to determine whether NE increases acetyllysine modifications of Histone H3 and HMGB1 as part of the mechanism of increased inflammation, and 2) identification of the proteome of NE-activated METs in healthy volunteers and subjects with COPD.

15. SUBJECT TERMS

Neutrophil elastase, Macrophage, extracellular traps, HMGB1, histone deacetylase

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRDC
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TABLE OF CONTENTS

1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

The central hypothesis of this proposal is that extracellular NE is taken up by macrophages and accumulates in both cytoplasmic organelles and the nucleus. NE activity degrades histone deacetylase 2 (HDAC2) and possibly other HDACS and Sirtuins resulting in increased acetylation of several targets including histone H3, High Mobility Group Box 1 (HMGB1) and nuclear factor kappa B (NFkB) p65, resulting in increased cytokine transcription and release of HMGB1 (**AIM 1**). Nuclear NE cleaves histone H3 and increases H3 citrulline resulting in chromatin decondensation and release of vital nuclear METs (**AIM 2**).

2. **KEYWORDS:** *Provide a brief list of keywords (limit to 20 words).*

Chronic obstructive pulmonary disease, macrophages, human blood monocyte derived macrophages (hBMDM), extracellular traps, histone deacetylase, sirtuin, High Mobility Group Box 1, Nuclear factor kappa B, Neutrophil elastase (NE)

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

What were the major goals of the project?

List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

Major Task 1: Neutrophil elastase localization and protease activity; Cytokine mRNA and protein expression
Major Task 2: HDAC and Sirtuin expression and activity will be determined in hBMDM. Impact of loss of HDAC will be evaluated by identification of lysine acetylation of targets Histone H3, NFkB p65, or High Mobility Group Box 1 (HMGB1). To confirm impact of loss of HDACs/ Sirtuins, siRNA silencing of HDAC and/ or Sirtuin top candidates will be performed to test the impact on cytokine and HMGB1 release. Alveolar macrophages will be isolated and characterized to measure NE uptake, protease activity and HDAC or H3 modifications
Major Task 3: Quantitate DNA released into culture media; Determine nuclear H3 degradation, H3 citrulline, and PAD1-4 expression; Identify cationic protein candidates in conditioned media that are associated with METs by LC-MS.

What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

1) Major Activities:

Major Task 1. We plan to better characterize hBMDM polarization post-NE treatment by evaluating cytokine release by MSD multiplex platform from hBMDM from subjects with COPD and isolating conditioned media post-NE and post- treatment with LPS or with IL-4/ IL-13 as demonstrated in Figure 1.

Major Task 2. We will confirm our initial results on NE-induced degradation of HDACs and Sirtuin 1 using additional healthy control hBMDM and using hBMDM from subjects with COPD to determine the impact of NE on these cells. We are collaborating with Dr. Hawkridge, who is establishing proteomic work-flow for cell lysates from healthy hBMDM to determine acetylated lysine modification of targets including HMGB1 and Histone H3.

Major Task 3. In collaboration with Dr. Hawkridge, we are establishing methods to enrich METs from healthy hBMDM treated with NE so that we have sufficient MET-associated protein to perform proteomic analysis of METs following NE treatment. We will confirm MET constituents in METs from hBMDM derived from subjects with COPD.

3) Significant Results and Key Outcomes

Major Task 1. We have completed NE localization and proteinase activity experiments in human blood monocyte derived macrophages (hBMDM). We recently examined whether hBMDM developed features of M1 or M2 polarization after NE treatment. We determined that NE treatment caused increased TNFα release from hBMDM consistent with M1 polarization, but no CCL18, consistent with no M2 polarization. In contrast treatment of the hBMDM with IL-4 and IL-13 caused increased CCL18 release but little to none TNFα release, demonstrating that these cells are capable of M2 polarization under control treatment conditions (**Figure 1**).

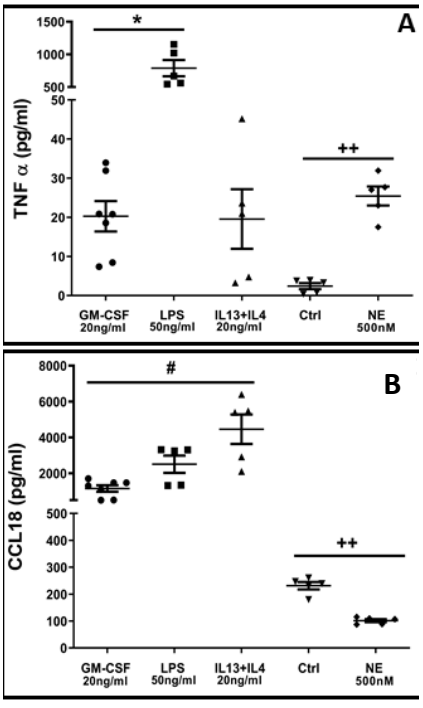


Figure 1. Human BMDM exhibited M1 like phenotype post- NE exposure as determined by cytokine secretion. Monocytes obtained from healthy subjects were differentiated to macrophages in primary culture with GM-CSF (10 days). These cells alone or after stimulation with NE (500nM) or control vehicle for 2h were evaluated for cytokine expression in the conditioned media to determine M1 or M2 polarization. Quantitation of cytokines was performed by ELISAs: M1 cytokine, TNFα, in the conditioned media (A, data combined from 2 donors) and M2 cytokine, CCL-18, in the conditioned media (B, data combined from 2 donors). To generate positive controls for M1 and M2 macrophage differentiation, monocytes were cultured for 6 days with GM-CSF, and then treated with LPS (50ng/ml) for 4 days to generate M1 BMDM, or treated with IL-4 (20ng/ml) + IL-13 (20ng/ml) for 4 days; with media changes after 2 days. Conditioned media reflected two days of culture due to the treatment conditions from GM-CSF -differentiated BMDM cells, and the positive control cells for M1 (LPS treated) and for M2 (IL-4 and IL-13 treated). In contrast, conditioned media from control-treated and NE-treated cells reflected only contribution from 2 h of culture. NE treated cells had increased TNFα compared to control treated cells, but lower CCL-18 compared to control treated cells, consistent with M1 phenotype. The results are summarized (mean ± SEM) from 2 experiments, n= 6-8 replicates. Statistically significant differences between NE and control treatment were determined by ANOVA with post-hoc comparisons by the Wilcoxon rank sum test: ++, $p < 0.008$ vs Ctrl treated. Statistically significant differences between M1-polarized and M2-polarized cells vs. GM-CSF treated cells for TNFα expression (*, $p<0.025$); and for CCL-18 expression (#, $p <0.02$, vs GM-CSF treated) are shown.

Major Task 2. Major Task 2: HDAC and Sirtuin expression and activity will be determined in hBMDM. We have completed western analysis of all HDAC and Sirtuin protein expression profiles after treatment of hBMDM with different dose of NE (200 & 500nM) or control vehicle for 2 h from

two separate healthy control hBMDM. Results are shown in **Figure 2** for HDAC Class 1, in **Figure 3** for HDAC Class II, and in **Figure 4** for Sirtuin 1 (HDAC Class IV). To improve the rigor of these results, we will complete a third healthy hBMDM donor to ensure that our results are consistent. We demonstrate that NE degraded HDAC activity in a dose-dependent manner and caused a small decrease in histone acetyltransferase activity (**Figure 5**). We also demonstrated that the resulting unopposed acetyltransferase activity resulted in translocation of High Mobility Group Box 1 (HMGB1) from the nucleus to the cytosol (**Figure 6**). We confirmed this mechanism by treating hBMDM with a global HDAC inhibitor, Trichostatin A (TSA), and demonstrating that HMGB1 translocated from the nucleus to the cytosol (**Figure 7**), a prerequisite for extracellular release of HMGB1.

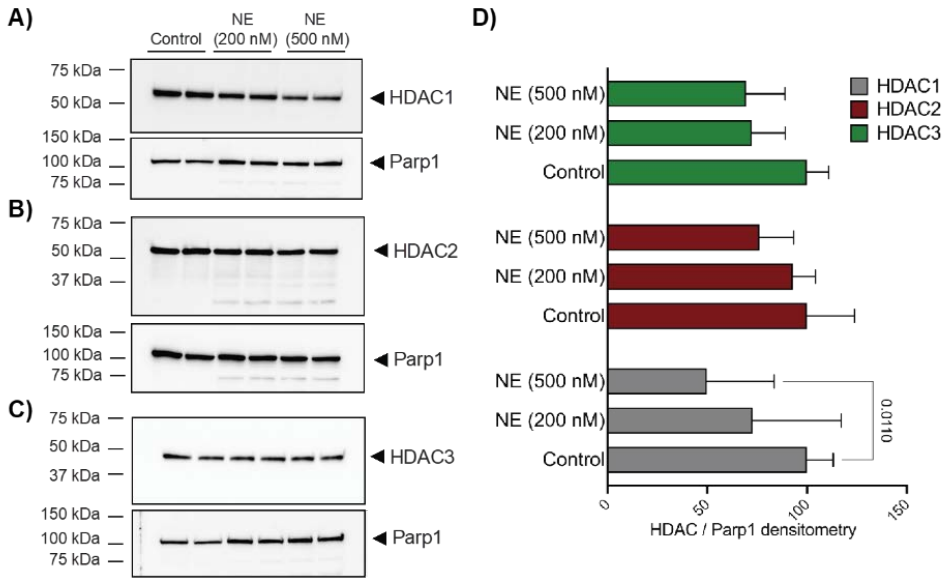


Figure 2. NE-treatment of hBMDM degraded nuclear HDAC 1-3 (Class 1). Following NE (200-500 nM) vs control treatment, 2 h of hBMDM from control subject buffy coat- derived cells, nuclei were isolated by cell fractionation and nuclear protein lysates were harvested, quantified and used for western analysis. Western blotting was performed using antibodies against HDAC1 (Santa Cruz sc-81598, 1:500 in 5% milk), HDAC2 (Cell Signaling Technologies #5113s, 1:1000 in 5% milk), HDAC3 (Cell Signaling Technologies #3949s, 1:1000 in 5% milk), PARP1 (Santa Cruz sc-8007, 1:500 in 5% milk). Band intensities were quantified using ImageLab (Bio-Rad). P=0.011 for HDAC1 comparing Control vs NE (500nM) using Two-way ANOVA in GraphPad Prism, n=2 donors, n=2 replicates for each donor sample.

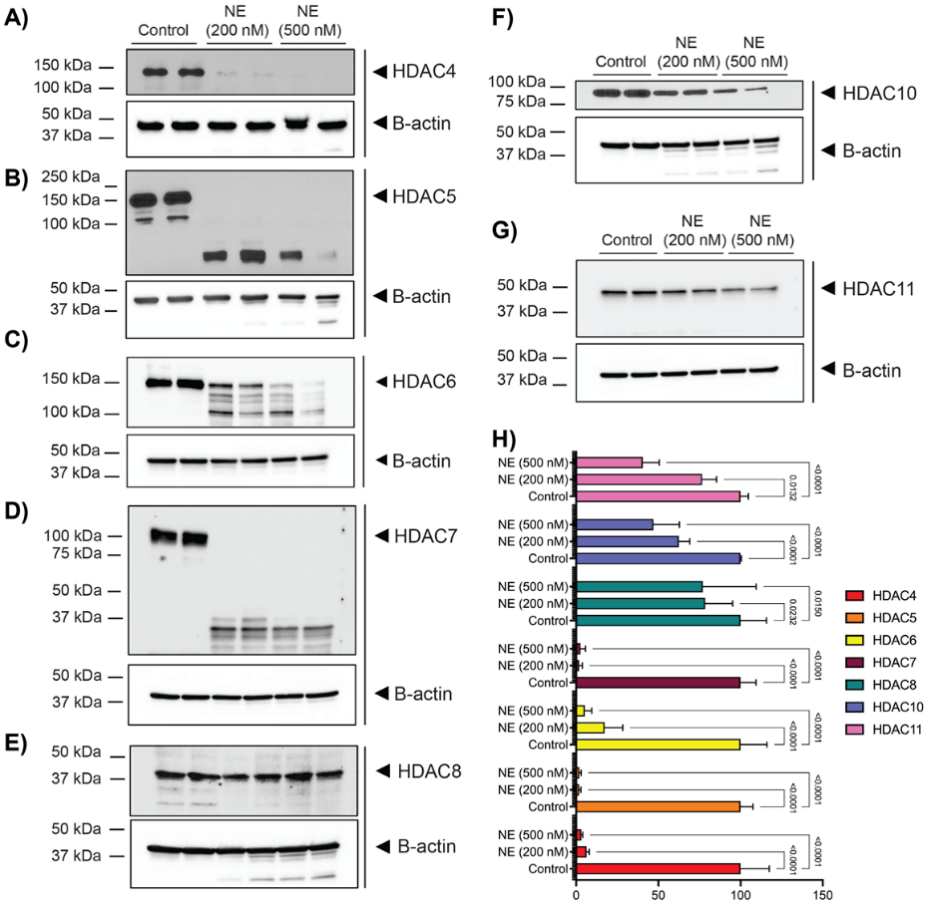


Figure 3. NE treatment of hBMDM degraded HDAC 4-11 (Class II) in the cytosol. Western blotting was performed using antibodies against HDAC4 (Santa Cruz sc-46672, 1:500 in 5% milk), HDAC5 (Santa Cruz sc-133106, 1:500 in 5% milk), HDAC6 (Cell Signaling Technologies #7558s, 1:2000 in 5% milk), HDAC7 (Santa Cruz sc-74563, 1:500 in 5% milk), HDAC8 (Santa Cruz sc-374180, 1:500 in 5% milk), HDAC10 (Santa Cruz sc-393417, 1:500 in 5% milk), HDAC11 (Santa Cruz sc-390737, 1:500 in 5% milk), B-actin (Sigma A5441, 1:8000 in 5%milk). Secondary antibodies were used at (1:2000-1:4000) either CST #7076, or CST #7074 or NXA931 (1:10,000 for B-actin) Band intensities were quantified using ImageLab (Bio-Rad) or Fiji for scanning of X-ray films. Two-way ANOVA in GraphPad Prism, n=2 donors, n=2 replicates for each donor sample.

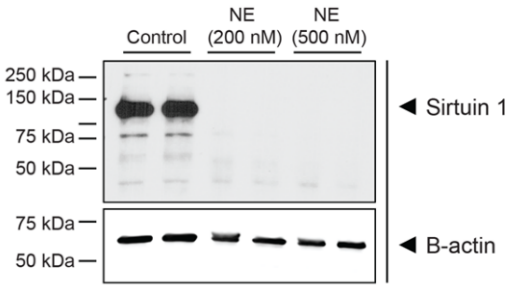


Figure 4. NE significantly decreased Sirtuin 1 in hBMDM. Cells were treated with either control vehicle or Neutrophil Elastase as indicated. Total cell lysates were prepared using (Cell Signaling Technologies Cell Lysis Buffer) or nuclear extracts were prepared for (Sirtuin 6 and Sirtuin 7), and equal amounts of protein were separated using SDS-PAGE. Western blotting was performed using antibodies against Sirtuin Antibody sampler kit (Cell Signaling Technologies, 1:1000). Secondary antibodies were used at (1:2000-1:4000) either CST #7076, or CST #7074 or NXA931 (1:10,000 for B-actin). Band intensities were quantified using ImageLab (Bio-Rad) or Fiji for scanning X-ray films. Two-way ANOVA in GraphPad Prism, n=1 donor, n=2 replicates for each donor sample**.**

Figure 5. Global HDAC activity was decreased upon NE treatment. hBMDM were incubated with HDAC Glo asay reagent (G6420, Promega) to detect HDAC activity per manufacturer's instructions. Global HAT Assay kit (K332, Biovision) was performed using 45ug of nuclear extract samples isolated from hBMDM cells after control vehicle or NE treatment. DTT was omitted during nuclear extract preparation. (n=3 donors, 2 replicates for each donor); *, $p<0.05$, ****, $p<0.001$.

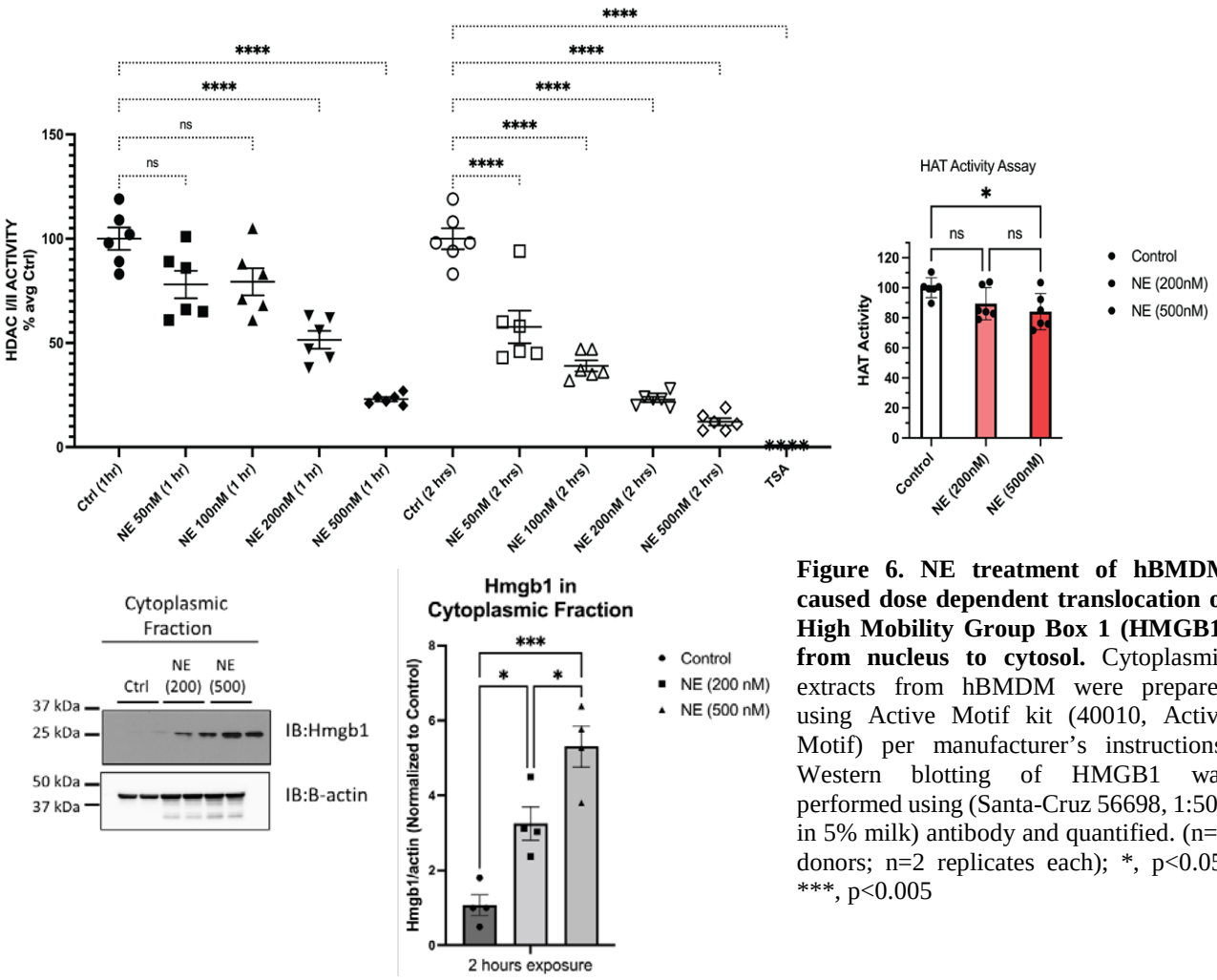


Figure 6. NE treatment of hBMDM caused dose dependent translocation of High Mobility Group Box 1 (HMGB1) from nucleus to cytosol. Cytoplasmic extracts from hBMDM were prepared using Active Motif kit (40010, Active Motif) per manufacturer's instructions. Western blotting of HMGB1 was performed using (Santa-Cruz 56698, 1:500 in 5% milk) antibody and quantified. (n=2 donors; n=2 replicates each); *, $p<0.05$; ***, $p<0.005$

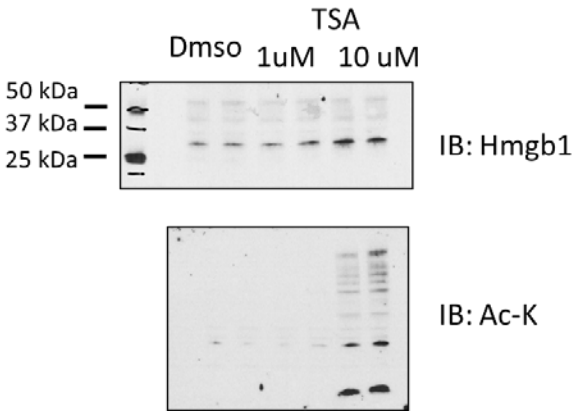


Figure 7. TSA treatment of hBMDM increased cytosolic translocation of HMGB1 and proteins with acetylated lysine. Cytoplasmic extracts from hBMDM were prepared using Active Motif kit (40010, Active Motif) per manufacturer's instructions. Western blotting of HMGB1 was performed using (Santa-Cruz 56698, 1:500 in 5% milk) antibody and quantified. Western analysis for acetylated lysine was performed using Rabbit Ac-K Ab (CST 9814S, 1:1000 dilution in 5%BSA and secondary HRP-conjugate anti-rabbit Ab, and ECL development. Western blot shown for one donor in duplicate samples.

Major Task 3: **Quantitate DNA released into culture media; Determine nuclear H3 degradation, H3 citrulline, and PAD1-4 expression.** We have already completed analysis of H3 clipping, H3 citrullination and determined that H3 clipping is due to NE activity but H3 citrullination is not related to changes in protein abundance of PAD2 or 4.

Table 1. COPD subject demographics for NE-induced METS analysis

Sex		Race		Smoking Status		COPD Gold Score	
Female	Male	AA	W	Active	Past	Mild (A,B)	Severe (C,D)
5	9	4	10	5	9	9	5

Human BMDM were obtained from subjects with COPD and demographic characteristics were summarized in **Table 1**. Cells were exposed to NE (50-500 nM) or control vehicle for 2 h, conditioned media removed, and cells incubated with micrococcal nuclease (MN, 16 U/ml, 20 min) to release METs into the MN conditioned media. Quantitation of extracellular double strand DNA (ecDNA) was performed using the Quanti-IT ® PicoGreen assay (**Figure 8**). NE triggered release of ecDNA occurred after exposure of 50 nM NE or greater (**Figure 8A**). When comparing subjects with different demographic characteristics of sex and disease severity, women (**Figure 8B**) and subjects with increased disease severity (**Figure 8C**) released greater quantity of METs at higher NE concentrations.

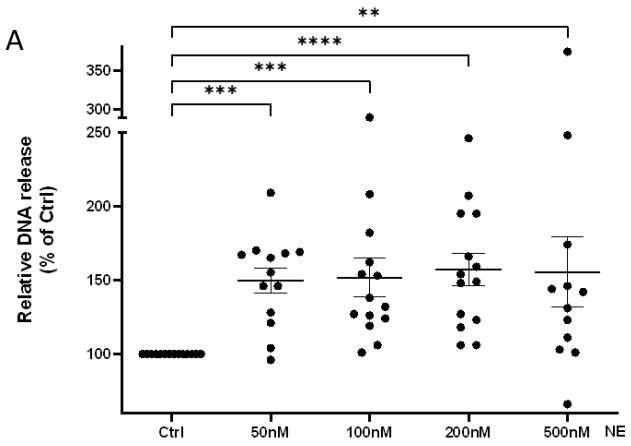
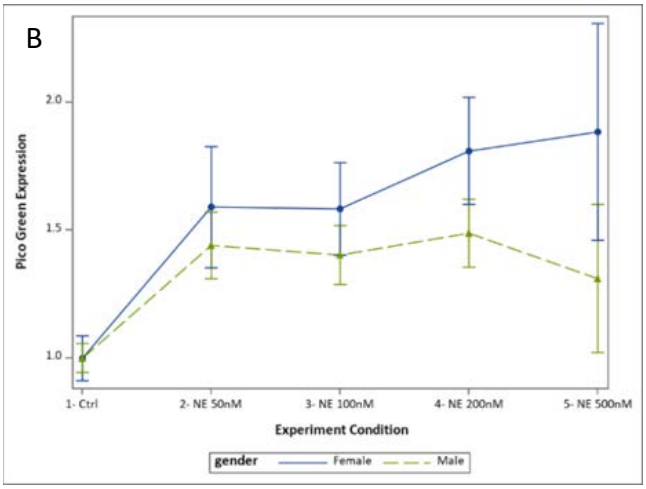
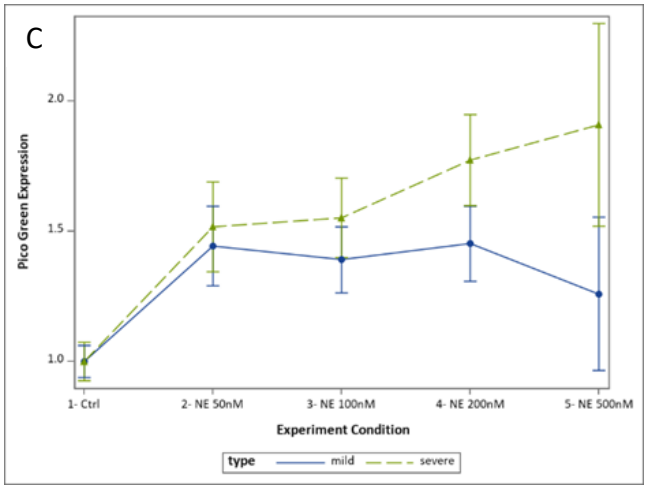


Figure 8. NE induced the release of METs as indicated by ecDNA in COPD hBMDM. Following NE and control vehicle treatments, 2h, 37°C, conditioned media was removed and cells were incubated with micrococcal nuclease and the supernatant containing ecDNA released (MET release) was quantified by Quant-iT PicoGreen dsDNA Assay kit and compared to control treated cells (**A**). N=14; data normalized to average control; mean ± SEM; **, p<0.01; ***, p<0.001; ****, p<0.0001. Women (B) had greater MET release than men and subjects with severe disease (C) had greater disease than those with mild disease.





What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Nothing to Report.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.
The results from this project are being presented by poster presentations:

1. Abstract: Kummarapurugu et al, Neutrophil elastase activates the release of extracellular traps from COPD monocyte-derived macrophages, Thomas Petty Lung Conference, June 2022, Aspen, CO [*manuscript in preparation*].
2. Abstract: Voynow et al, Neutrophil elastase–mediated degradation of histone deacetylases in macrophages promotes high mobility group box 1 export from nucleus to cytosol, NACFC meeting, November 2022, Philadelphia, PA [*manuscript in preparation*].

Three manuscripts have been published that are relevant to this project:

1. Kummarapurugu, AB, Zheng, S, Ma, J, Ghosh, S, Hawkridge, A, and Voynow, JA, Neutrophil Elastase Triggers the Release of Macrophage Extracellular Traps, Relevance to Cystic Fibrosis, 2022, Am. J. Respir. Cell Mol. Biol., 66: 76-85, PMID: 34597246; PMCID: PMC8803356
2. Voynow, JA and Shinbashi M, Neutrophil Elastase and Chronic Lung Disease, 2021, Biomolecules, 11:1065, PMID: 34439732; PMCID: PMC8394930; DOI: 10.3390/biom11081065
3. Voynow, JA, Zheng, S, Kummarapurugu, AB, Glycosaminoglycans as Multifunctional Anti-Elastase and Anti-Inflammatory Drugs in Cystic Fibrosis Lung Disease, 2020, Front. Pharmacol., 11: 1011, PMID: 32733248; PMCID: PMC7360816; DOI: 10.3389/fphar.2020.01011

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

We are working closely with Dr. Hawkridge who has a graduate student dedicated to this project to determine 1) the NE-induced acetylated lysine proteome in the hBMDM cell lysates and compare healthy to COPD differences in acetylated lysine modifications; and 2) the MET proteome and whether it differs between subjects with COPD, CF or healthy volunteers. They are developing the Proteome methods and standards and we are preparing the cell lysate or MET biospecimens for analysis.

4. **IMPACT:** D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

We made the unprecedented discovery that Neutrophil elastase (NE) is a potent stimulus that drives macrophages into a pro-inflammatory phenotype triggering the release of extracellular traps (MET). This novel observation shifts attention to METs as structures that propagate lung inflammation in patients with COPD. Importantly, chronic lung inflammation persists and progresses in patients with COPD. To date, there are no therapies that interrupt the progressive injury in the lungs of patients with COPD, that ultimately results in lung failure. METs potentially present a new therapeutic target to mitigate lung inflammation in COPD.

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Neutrophil elastase is a major inflammatory mediator in cystic fibrosis, bronchopulmonary dysplasia, pneumonia and ARDS. We reported that the mechanism for NE-triggered MET release is relevant to the CF airway (Kummarapurugu (2022) Am. J. Respir. Cell Mol. Biol.) and NE-triggered MET release may be an important component of lung inflammation in the other chronic lung diseases. We are currently developing the technology to define the components of METs in specific chronic lung diseases and how they propagate downstream inflammation in the lung.

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to Report

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to Report

5. **CHANGES/PROBLEMS:** *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

Nothing to Report

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

We had delays in recruiting subjects due to COVID19 closure of outpatient in person COPD clinics. We also had a delay in proteomic analyses due to personnel leaving from the Proteomic program and the need to replace and train the graduate student now working on this project.

Changes that had a significant impact on expenditures

Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Nothing to Report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Nothing to Report

Significant changes in use or care of vertebrate animals

Nothing to Report

Significant changes in use of biohazards and/or select agents

Nothing to Report

6. PRODUCTS: *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Three manuscripts have been published that are relevant to this project: 1. Kummarapurugu, AB, Zheng, S, Ma, J, Ghosh, S, Hawkridge, A, and Voynow, JA, Neutrophil Elastase Triggers the Release of Macrophage Extracellular Traps, Relevance to Cystic Fibrosis, 2022, Am. J. Respir. Cell Mol. Biol., 66: 76-85, PMID: 34597246; PMCID: PMC8803356 2. Voynow, JA and Shinbashi M, Neutrophil Elastase and Chronic Lung Disease, 2021, Biomolecules, 11:1065, PMID: 34439732; PMCID: PMC8394930; DOI: 10.3390/biom11081065 3. Voynow, JA, Zheng, S, Kummarapurugu, AB, Glycosaminoglycans as Multifunctional Anti-Elastase and Anti-Inflammatory Drugs in Cystic Fibrosis Lung Disease, 2020, Front. Pharmacol., 11: 1011, PMID: 32733248; PMCID: PMC7360816; DOI: 10.3389/fphar.2020.01011

  Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to Report

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

The results from this project are being presented by poster presentations:
1. Abstract: Kummarapurugu et al, Neutrophil elastase activates the release of extracellular traps from COPD monocyte-derived macrophages, Thomas Petty Lung Conference, June 2022, Aspen, CO [manuscript in preparation].
2. Abstract: Voynow et al, Neutrophil elastase–mediated degradation of histone deacetylases in macrophages promotes high mobility group box 1 export from nucleus to cytosol, NACFC meeting, November 2022, Philadelphia, PA [manuscript in preparation].

- **Website(s) or other Internet site(s)**

List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to Report

- **Technologies or techniques**

Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

Nothing to Report

- **Inventions, patent applications, and/or licenses**

Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to Report

- **Other Products**

  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

  - *data or databases;*
  - *physical collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*
  - *instruments or equipment;*
  - *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
  - *clinical interventions;*
  - *new business creation; and*
  - *other.*

Nothing to Report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of

compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Example:

Name: *Mary Smith*
Project Role: *Graduate Student*
Researcher Identifier (e.g. ORCID ID): *1234567*
Nearest person month worked: *5*

Contribution to Project: *Ms. Smith has performed work in the area of combined error-control and constrained coding.*
Funding Support: *The Ford Foundation (Complete only if the funding support is provided from other than this award.)*

Name: Judith Voynow, MD
Project Role: Principal Investigator
Nearest Person month worked: 2
No change

Name: Shuo Zheng, PhD
Project Role: Co-Investigator
Nearest Person month worked: 6
No change

Name: Apparao Kummarapurugu, PhD
Project Role: Co-Investigator
Nearest Person month worked: 6
No change

Name: Le Kang, PhD
Project Role: Co-investigator
Nearest person month worked: 1
Contribution to Project: Statistical analysis of MET release intracellular protease activity

Name: Adam Hawkridge, PhD
Project Role: Co-investigator
Nearest person month worked: 3
Contribution to Project: HPLC-MS/MS analysis of MET constituents and acetylated lysine modifications of cytoplasmic proteins

Name: Erica Memoli
Project Role: Clinical Research Coordinator
Nearest person month worked: 4
Contribution to Project: Screening, recruiting and enrolling participants

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Nothing to Report

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
<u>Organization Name:</u>
<u>Location of Organization: (if foreign location list country)</u>
<u>Partner's contribution to the project</u> (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to* ***https://ebrap.org/eBRAP/public/index.htm*** *for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil/Pages/Resources.aspx) should be updated and submitted with attachments.*

9. APPENDICES: *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.*

Aspen Lung Conference 64th Annual Meeting, June 8-11, 2022

NEUTROPHIL ELASTASE ACTIVATES RELEASE OF EXTRACELLULAR TRAPS FROM COPD MONOCYTE-DERIVED MACROPHAGES

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Supported by DOD Congressionally Directed Medical Research Programs (CDMRP) Peer Reviewed Medical Research Program (PRMRP) PR180925 (JAV)

Introduction: The progression of COPD lung disease is due in part to failure of macrophage innate immune function. High concentrations of neutrophil elastase (NE) in the airway perpetuate macrophage dysfunction, but the mechanisms are not fully elucidated. We hypothesized that NE is taken up by macrophages and intracellular proteolytic activity results in the release of macrophage extracellular traps (METs), a DNA web-like structure with pro-inflammatory granule and chromatin binding proteins.
Methods: After IRB-approved, informed written consent, we obtained whole blood from 7 subjects with COPD. Monocytes were isolated and underwent primary culture to differentiate into blood monocyte-derived macrophages (bMDM). Blood MDMs were evaluated for NE uptake using FITC-labeled NE and immunofluorescence. Total extracellular DNA release from NE-treated bMDM was evaluated by a

fluorescent assay (Picogreen) and by confocal microscopy for DAPI-stained extracellular trap structures. We tested whether NE upregulated peptidyl arginine deiminase enzymes (PAD) 2 or 4 to induce citrullination of histones or proteolytically clipped histones, prerequisites for MET formation.
Results: FITC-NE was taken up by bMDM into the nucleus and the cytoplasm. NE uptake was confirmed using a primary anti-NE antibody and secondary rhodamine antibody. MET release occurred only after NE exposure and was quantified by Picogreen assay and confocal microscopy. NE did not impact PAD2 or 4 abundance, and did not affect Histone H4, but did cause Histone H3 clipping in a dose- dependent manner.
Conclusion: NE is taken up by bMDM and activates release of METS via Histone H3 clipping**.**

North American Cystic Fibrosis Conference, 11/2-5/2022, Philadephia, PA
Category: AIRWAYS PHYSIOLOGY, PATHOPHYSIOLOGY & AIRWAYS DEFENSE
Neutrophil elastase–mediated degradation of histone deacetylases in macrophages promotes high mobility group box 1 export from nucleus to cytosol
J. Voynow[1], G. Bulut[2], S. Zheng[2], A. Kummarapurugu[2]
[1]Pediatric Pulmonology, Children's Hospital of Richmond at Virginia Commonwealth University, Richmond, VA
[2]Department of Pediatric Pulmonary Medicine, Children's Hospital of Richmond at Virginia Commonwealth University, Richmond, VA USA

Background: Neutrophil elastase (NE) induces the release of high mobility group box 1 (HMGB1), a damage-associated molecular pattern (DAMP), from macrophages, but the mechanism of NE-induced release of HMGB1 is not known. HMGB1 is present in the nucleus as a chromatin-binding protein, but after lysine acetylation, it is retained in the cytosol and is fated for cellular release. We hypothesize that NE alters the epigenetic program of macrophages by degrading histone deacetylases, resulting in unopposed histone acetyltransferase activity, greater HMGB1 lysine acetylation, and greater cytosolic HMGB1 poised for cell export into the extracellular milieu.
Methods: Human blood monocytes were isolated from buffy coats obtained from deidentified healthy subjects from the American Red Cross and cultured with growth media containing granulocyte-macrophage colony-stimulating factor to differentiate into human blood monocyte-derived macrophages (hBMDMs), which were exposed to control vehicle or NE (200 or 500 nM) for 2 hours at 37°C. Nuclear protein or total cell lysate protein was isolated for western blot analysis to determine relative abundance of histone deacetylase (HDAC) Class I (1,2,3,8), Class II (4,5,6,7,8,9,10), Class III (Sirtuins 1-7), and Class IV (HDAC11). Cytosolic lysate protein harvested after control vehicle or NE treatment was used for western blot analysis for HMGB1. Western blots were normalized to β-actin (total cell lysate or cytosolic fraction) or poly (ADP ribose) polymerase 1 (nuclear fraction).
Results: NE treatment resulted in significantly greater cytosolic HMGB1 than found in control-treated cytosolic lysates. NE treatment significantly decreased Class II HDACs and Sirtuin 1. NE treatment also decreased Class I HDACs, but the change was not as pronounced as that observed for the Class II HDACs and Sirtuin 1.
Conclusions: Active NE taken up by hBMDMs degraded HDACs and Sirtuin 1, shifting HMGB1 localization from nucleus to cytosol, a priming event for HMGB1 release.
Acknowledgements: This work was supported by the Cystic Fibrosis Foundation (VOYNOW19G0), Department of Defense Peer-Reviewed Medical Research Program PR180925 (JAV), and National Institutes of Health R01 HL146811-01A1 (JAV)