PERIOSTEAL LESIONS AS MARKER FOR SYSTEMIC INFLAMMATORY SHIFTS IN TUBERCULOSIS AND LEPROSY INFECTIONS: AN *IN VITRO* ANALYSIS

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ABSTRACT

It is possible that during long lasting chronic infections such as tuberculosis (TB) and leprosy (LP) those individuals that generate a stronger immune response will produce a shift in the systemic levels of inflammatory mediators, heading to a potential hyper-inflammatory state or hyper-inflammatory phenotype (HIP) while fighting a chronic infection. Consequently, the systemic immunological shift could affect other persistent infections such as the one observed in periosteal lesions where the most common pathogen detected is *Staphylococcus aureus*. The objective of this study is to determine if *in vitro* immune cells exposure to *Mycobacterium tuberculosis* or *M. leprae* lysates impacts subsequent immune responses to persistent/local pathogen *S. aureus*. During a two-day experiment, we exposed human peripheral blood mononuclear cells (PBMCs) to either *M. tuberculosis* or *M. leprae* lysates on day one; sequentially on day two, we exposed the same culture to *S. aureus.* The expression of key proteins (TNFα and IFNγ) involved in the immune response was measured by ELISA. Preliminary results showed that early exposure (day 1) to LP lysate induces higher IFNγ expression when the same cells are exposed to *S. aureus*. Interestingly, early exposure to *S. aureus* altered IFNγ expression when cells subsequently were exposed to TB or LP lysates. These preliminary results show an immunological alteration when PBMCs are alternatively exposed to two different pathogens. These findings could be useful in osteological analyses when considering how TB or leprosy infection can have effect on other osteological lesions through the promotion of a HIP.

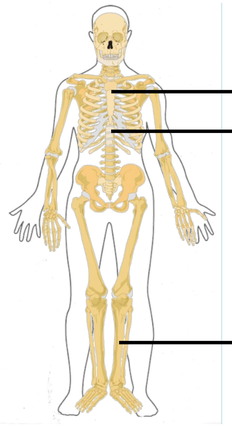
BACKGROUND

The immunological competence of an individual is a reflection of its evolutionary history, and different infectious diseases play a significant role in constantly reshaping the immune response. While the immune system protects us from pathogens, many of its mechanisms are also major contributors to tissue damage and disease. Deficient responses may result in increased susceptibility to pathogens while excessive ones may result in heightened inflammation and pathologic consequences to the host. Immune and inflammatory responses are known to be regulated by multiple factors, including cytokines which are soluble mediators of the immune system. Pathogens can overcome the host immune response by different mechanisms such as immunosuppression, though some individuals will be able to mount a stronger immune response (commonly associated with hyper-inflammation) that can overcome the immunosuppression generated by some pathogens. Therefore, it is possible that during long lasting chronic infections such as tuberculosis (TB) and leprosy (LP) those individuals that generate a stronger immune response will produce a shift in the systemic levels of inflammatory mediators, heading to a potential hyper-inflammatory state or hyper-inflammatory phenotype (HIP) while fighting a chronic infection. Consequently, the systemic immunological shift could affect other persistent and regular chronic infections such as the ones generated by *Staphylococcus aureus* commonly associated with periosteal lesions. While pro-inflammatory proteins, such as tumor necrosis factor alpha (TNFα) and interferon gamma (IFNγ), enhance the cellular response against pathogens (i.e. *S. aureus*), they could also induce excessive osteoclastogenesis and bone remodeling or destruction; therefore the immunological shift generated by co-infections such as TB and LP should be taken into consideration when analyzing, for example, periosteal lesions as well as other osteological lesions in skeletal remains. Studying the immunological shift generated by chronic infections such as TB and LP in living populations posits a logistic and experimental problem, therefore our first step is to use novel *in vitro* experimental protocols to study systemic inflammation and its potential consequences on persistent/local infections.

OBJECTIVE

The objective of this study is to determine if *in vitro* immune cells exposure to *Mycobacterium tuberculosis* or *M. leprae* lysates impacts subsequent immune responses to persistent/local pathogen *S. aureus.*

RATIONALE



Leprosy

Tuberculosis

Systemic hyper-inflammation?

A. **High expression** of pro inflammatory cytokines: **TNFα** and **IFNγ**

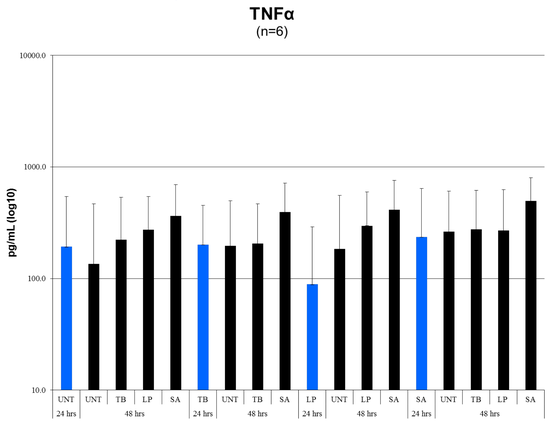
B. Does the higher expression of inflammatory cytokines generate a systemic shift? = **HYPER-INFLAMMATORY STATUS (HIS)**

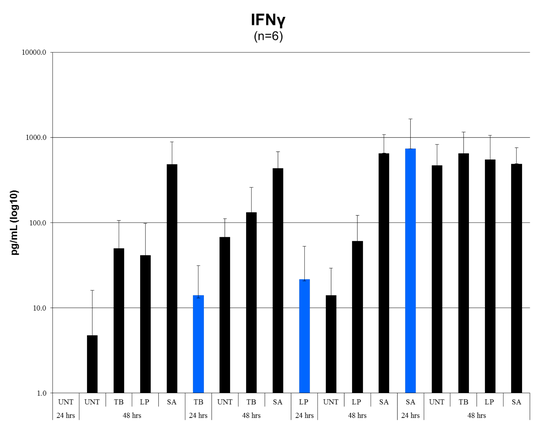
C. Does HIS **affect inflammatory responses** against other **persistent infections** such as the one present in periosteal lesions (commonly associated with *S. aureus)?*

Rationale for preliminary analysis to determine if ***in vitro*** exposure to ***M. tuberculosis*** or ***M. leprae*** lysates impacts subsequent immune responses to persistent/local pathogens ***S. aureus.***

RESULTS

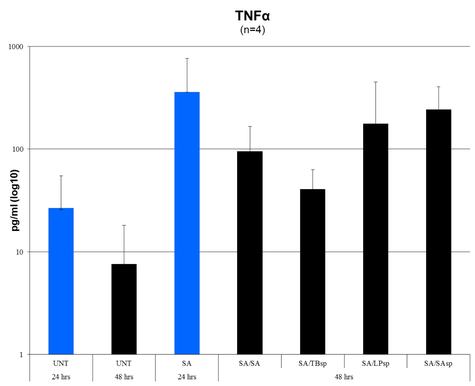
PROTOCOL 1: Does IFNγ and TNFα expression by PBMCs exposed to *S. aureus* change if the **same cells** have an early exposure to *M. tuberculosis* or *M. leprae*?

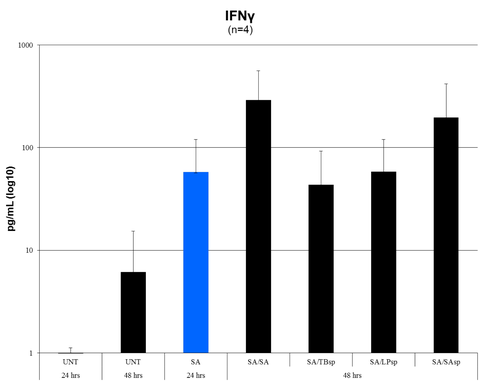




UNT: untreated; TB: *M. tuberculosis* lysate; LP: *M. leprae* lysate; SA: *S. aureus* lysate

PROTOCOL 2: Do **factors released** by PBMCs exposed to *M. tuberculosis or M. leprae* affect IFNγ and TNFα expression by naïve PBMCs (or ones exposed to *S. aureus)?*





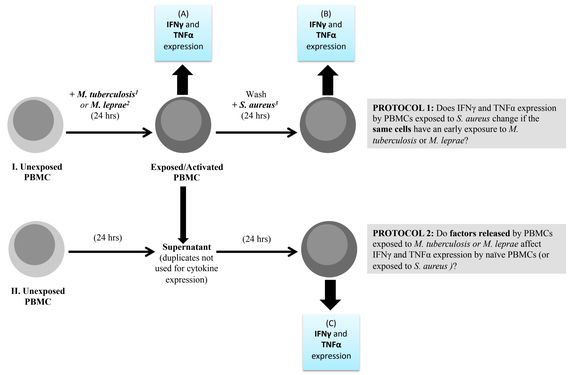
UNT: untreated; SA: *S. aureus* lysate; TBsp: supernatant from cells exposed to *M. tuberculosis* lysate; LPsp: supernatant from cells exposed to *M. leprae* lysate; SAsp: supernatant from cells exposed to *S. aureus*

MATERIALS and METHODS

- Blood extraction (healthy donors)

PERIPHERAL BLOOD MONONUCLEAR CELLS (PBMCs)

Cells were cultured with RPMI-10%FCS in 24 well plates under (i.e.) the following conditions:



PROTOCOL 1: Does IFNγ and TNFα expression by PBMCs exposed to *S. aureus* change if the **same cells** have an early exposure to *M. tuberculosis or M. leprae* affect IFNγ and TNFα expression by PBMCs exposed to *M. tuberculosis or M. leprae* affect IFNγ and TNFα expression by naïve PBMCs (or exposed to *S. aureus)?*

PROTOCOL 2: Do **factors released** by PBMCs exposed to *M. tuberculosis or M. leprae* affect IFNγ and TNFα expression by naïve PBMCs (or exposed to *S. aureus)?*

1: Whole Lysate from *Mycobacterium tuberculosis* (BEIResources - NR-14822)
2: Whole Lysate from *Mycobacterium leprae* (BEIResources - NR-19329)
3: Whole Lysate from *Staphylococcus aureus* (Pansorbin cells-Calbiochem # 507861, EMD Millipore, Billerica, MA)
Lipopolysaccharide from *E. coli* 0111:B4 (Sigma)

Cytokine expression (TNFα and IFNγ) in all supernatants was measured by enzyme-linked immunosorbent assay (ELISA) from eBioscience (San Diego, CA).

CONCLUSIONS

- Exposure to *S. aureus* lysate promotes the highest increase in TNFα and IFNγ expression in most conditions (24 hrs. or 48 hrs.).
- When comparing TNFα and IFNγ expression after *S. aureus* lysate exposure on day 2 but different lysate exposure on day 1 (*M. tuberculosis* or *M. leprae* or *S. aureus*); all three conditions show similar cytokine expression but slightly higher for IFNγ after earlier exposure to *M. leprae* lysate.
- When using supernatants (Protocol 2) from cells exposed to different pathogen lysates, TNFα showed similar expression when comparing SA/SAsp vs. SA/LPsp; however this was not observed in IFNγ (SA/SAsp > SA/LP).
- Overall, these preliminary results (with the current sample size) show that there is immune response alteration occurring in response to exposure to more than one pathogen; especially when sequentially exposed to *M. leprae*, *S. aureus*; or supernatant from activated cells by *M. leprae* or *S. aureus*.

FUTURE DIRECTION

- Using current protocols and considering the inter-sample variability (different donors), we plan to expand sample size to allow more power for future statistical analysis.
- We plan to explore the interaction of immune cells (supernatants) activated by all three lysates (*M. tuberculosis* or *M. leprae* or *S. aureus*) with bone cell differentiation (osteoclastogenesis); to allow for a more solid integration of experimental data and bioarchaeological studies.
- To explore different bioarchaeological data where as to assess the relationship between skeletal lesions caused by inflammatory processes observed in periosteal lesions, and osteological changes observed in individuals with tuberculosis or leprosy infection.