SOLUBLE MOLYBDENUM CAN INDUCE AN ADAPTIVE IMMUNE RESPONSE

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INTRODUCTION: Hypersensitivity to implant metals (e.g. Co, Cr, Mo and Ni) determined by Lymphocyte Transformation Testing (LTT), is found in 10 % of the general population, 25% of individuals with well performing total joint arthroplasties (TJA) and 60% of individuals with failing TJAs (1). While Delayed Type Hypersensitivity (DTH) responses in reactive subjects are attributed to common metal sensitizers such as Co, Ni and Cr, responses to other implant metals such as Mo have not been characterized. Molybdenum is 4.5-11% of the weight of Co-Cr-Mo alloy orthopedic implants (i.e. ASTMF75, ASTMF562). Although T cell structural recognition of metal-haptens remains to be elucidated for all metals, past reports indicate Be, Co and Ni, preferentially bind to MHC II alleles indicating a direct metal association with TCR and consequent recognition of specific T cells, i.e. a DTH response (2). Will other important implant metals like Al, Mo, and V elicit responses characteristic of DTH? We hypothesized that Mo specific reactivity will exhibit a delayed type hypersensitivity-like response where antigen presenting cells are required for Mo (haptenic form) presentation and activation of Mo-specific lymphocytes (HLA-dependent). To test our hypothesis we screened subjects for Mo reactivity until a Mo-reactive primary human lymphocyte cell line was found and assessed for proliferation, activation and cytokine production.

MATERIALS AND METHODS: *Lymphocyte proliferation:* 44 human subjects were screened for moderate Mo reactivity (Stimulation Index >3), until a reactive individuals cell were found to Mo. Peripheral blood mononuclear cells or isolated CD4+ T cells from Mo reactive human primary lymphocytes (1.5 x 10^5) were challenged with 0.01 mM and 0.1 mM $MoCl_2$ for 5 days. Proliferation was measured by [^{3}H]-thymidine (1mCi /well) incorporation for 5 days. *Immuno-staining:* Primary human Mo-reactive PBMCs were cultured with or without Mo ions (0.1 mM). PBMCs were double stained and analyzed each day following standard flow cytometry protocols with CD4-PE, CD69-FITC for T cell activation and CD19-PE, CD69-FITC for B cell activation. (PHA was used as a positive control for activation). *Mo-reactive T cell enrichment*: CD4+ T cells were negatively selected from a Mo-reactive human primary lymphocyte cell line with an antibody enrichment column following manufacturer protocols (R&D Systems). *Human 25-plex luminex cytokine analysis (IL-1alpha, IL-1beta, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12p40, IL-13, IL-15, IL-17, IFN-alpha, IFN-gamma, IP-10, Eotaxin, GM-CSF, MCP-1, MIP-1alpha, MIP-1beta, MIG, RANTES, and TNF-alpha):* Mo-reactive PBMC's and isolated CD4+ T cells were cultured with or without Mo ions (0.1 mM). Culture supernatants were collected at days 1, 2, 3, 4 and 5. Statistical significance was determined using t-testing, $p<0.05$.

RESULTS: Supporting our hypothesis, Mo-reactive lymphocytes showed increased proliferation, up-regulation of activation surface markers and pro-inflammatory cytokines in response to Mo ion challenge. Primary human Mo-reactive lymphocytes exhibited 2370.5 cpms compared to 498.75 cpms of their untreated controls at day 5 (Fig.1.A). Isolated Mo-treated CD4+ T cells (90% purity) did not show increased proliferation compared to untreated controls (Fig.1.B). Flow cytometry analysis of surface activation marker CD69 indicated an 8% increase of activated (CD4+ CD69+) T cells in PBMCs at day two of culture (Fig.1.C) Isolated CD4+ T cells did not show any increase in activation during 5 days of culture (Fig.1.D). B cells did not show an increase of activated cells in response to Mo at any time point tested (not shown). 25 different cytokines were assayed during 5 days of culture of Mo-reactive lymphocytes co-cultured as PBMCs or as isolated CD4+ T cells alone. GM-CSF, TNF-α m MIP1-β and IL-2r were elevated in the early stages of culture, decreasing to basal levels by day 5. GM-CSF and IL-2r showed the highest concentrations increasing up to 6.7 and 5.9 fold respectively. IFN-γ maintained basal levels throughout the experiment Fig.1.E). Isolated Mo-reactive CD4+ T cells alone were unable to induce significant differences in any of the cytokines tested (not shown).

DISCUSSION: The pattern of proliferation, surface marker expression and cytokine production indicated that Mo induced an antigen presenting cell dependent response of the TH1 type variety, all strong evidence of a DTH response. Previous studies have reported that Mo ion concentrations in subjects with well functioning total joint arthroplasties (TJA) are significantly elevated in synovial fluid (2.7 fold), the joint capsule (6.4 fold) and whole blood (5 fold) compared to normal subjects with no metal implants (1). Therefore, the increasing saturating concentrations of Mo could induce Mo-protein hapten formation resulting in immune activation in reactive subjects. The proliferation, activation and release of pro-inflammatory cytokines from Mo-reactive primary human lymphocytes (CD4+ T cells), co-cultured with APCs (autologous B-cells and monocytes PBMC), indicates a delayed type hypersensitivity where antigen-specific responses to Mo result in a TH1 type repertoire of cytokines without the characteristic release of IFN-gamma, suggesting there may be subtle additional specificity of response to specific metal-antigen reactivity. Blocking of MHCII presentation with a monoclonal antibody in challenged PBMCs prevented proliferation of APC-mixed Mo-specific lymphocytes. This further supports our hypothesis of Mo-protein antigen processing and presentation. While GM-CSF, TNF-α, IL-2r and MIP1-β were highly secreted, IFN-γ (prominent Th1 cytokine) was not detected. Th2 cytokines were also not detected with the exception of IL-10. Further study is warranted to further elucidate different cell subsets specific cytokine signaling effect in response to metal-protein antigen challenge.

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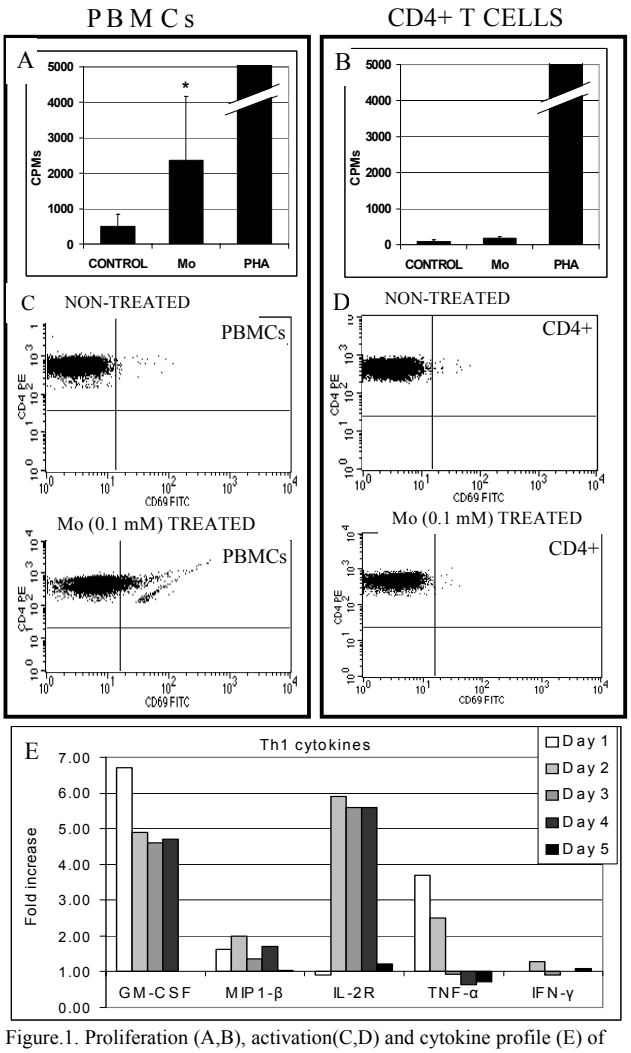

Figure.1. Proliferation (A,B), activation(C,D) and cytokine profile (E) of primary human Mo-reactive lymphocytes in response to Mo ions. Note: * = $p<0.05$