MHC-CLASS II EXPRESSION IN MACROPHAGES/MONOCYTES IS AFFECTED BY BOTH SOLUBLE AND PARTICULATE METAL IMPLANT DEBRIS

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INTRODUCTION: While the production of TNFα, IL-1β, IL-6, M-CSF and other cytokines have been reportedly secreted in response to metal particulate challenge, antigen processing and presentation of soluble and particulate debris have not been widely investigated. In order to generate lymphocyte mediated immune responses to metal-protein antigens (i.e. delayed type hypersensitivity), antigen presenting cells including but not limited to monocytes/macrophages must first uptake and degrade metal-protein antigens in endocytic compartments to present antigenic peptides on MHCII molecules (1). In general the activation of antigen presenting cells upon metal ion/particle phagocytosis induces cytokine secretion and broader mechanisms of activation that include surface expression of antigen presentation proteins and co-stimulatory molecules (i.e. MHCII, CD80, CD86) (2). We hypothesized that macrophages/monocytes will up-regulate surface expression of MHCII molecules in response to challenge with soluble and particulate debris. To test this hypothesis, we isolated primary human macrophages/monocytes and challenged them with or without soluble and particulate metals for 48 hours. Surface expression of MHCII molecules was measured with an indirect superantigen-pulsing method and by direct monoclonal antibody immunostaining analyzed by flow cytometry.

MATERIALS AND METHODS: PBMCs were isolated from healthy volunteers (n=6) by Ficoll gradient separation. PBMCs (1 x 10^6) were cultured in 48 well plates in Dulbecco's modified Eagle medium (GIBCO, Grand Island, N.Y) with 10% autologous serum at 37º and 0.5% Co2 for 24 hours. After 24 hours, non-adherent lymphocytes were removed and kept viable, leaving adherent monocytes (1.0x10^6) at the bottom of the wells (80 – 90 % purity). Adherent monocytes /macrophages were washed twice with PBS and challenged with or without 0.1 mM CoCl2, CrCl2, NiCl2, MoCl2 (Sigma, St Louis, MO) and Co-Cr-Mo alloy particles (mean diameter = 2um) (Bioengineering Solutions Inc, Chicago, IL) at a 10:1 ratio for 48 hours in fresh medium. *Toxic shock syndrome toxin 1 (TSST1) pulsing*: After metal challenge, monocytes/macrophages were washed twice with PBS and pulsed with 10mg/ml of TSST1 (binds MHCII molecules and TCR Vβ2 chain) for 2 hours, washed twice with PBS and co-cultured with autologous lymphocytes for 48 hours. Lymphocyte proliferation was measured by [3H]-thymidine (1mCi/ well) incorporation. *Flow cytometry*: Challenged monocytes /macrophages were immunostained with a mouse anti-human HLA-DR antibody and analyzed with standard flow cytometry protocols (Beckton-Dickenson). Results are shown as percent increase/decrease over non-treated controls.

RESULTS: Monocytes/macrophages challenged with soluble and particulate debris exhibited both increases and decreases of surface MHCII after 48 hours in a metal and subject-dependent fashion. NiCl2-treated monocytes/macrophages showed the lowest percentage change range in MHCII expression increasing up to 28.9% in subject 1 and decreasing -17% in subject 5 (Fig.1.A). The highest increase observed was 76.1% by MoCl2 treated cells in subject 4, while the biggest percentage loss was observed in subject 6 (-70.6%) when treated with CoCr particles. Subject 2 indicated percentage increases in all metals tested, whereas subject 6 indicated a percentage decrease in all metals tested (Fig.1.A). While CoCl2, NiCL2 and CoCr particles down-regulated MHCII in subject 5, CrCl2 and MoCl2 induced its up-regulation. Tetanus toxin only induced up-regulation of MHCII in 3 of 6 subjects tested. Flow cytometry analysis of MHCII expression indicated similar patterns of increased/decreased surface expression. There was an increased MHCII expression in response to CoCl2 and CrCl2 with a mean fluorescent intensity of 181.27 and 176.18 respectively compared to 156.68 of untreated controls (Fig.1.B.). A decrease in MHCII expression was observed in MoCl2 treated monocytes/macrophages that indicated an MFI of 119.53. NiCl2 only induced a small increase in MHCII with an MFI of 164.42. Results in Fig.1.A are expressed as lymphocyte cpm percentage increase/decrease over/under untreated controls and fluorescent intensity is represented by histograms on Fig.1.B.

DISCUSSION: The observed decreased surface expression of MHCII induced by certain metals in all subjects tested, refutes our original hypothesis, which stated that MHCII will be upregulated in macrophages in response to soluble and particulate metal challenge. Although up-regulation of MHCII in 5/6 subjects tested can be attributed to the normal activation and maturation of monocytes/macrophages, down-regulation of MHCII is a novel phenomena possibly caused by subject-specific intracellular protein interactions to soluble and particulate metal components that hinder MHCII localization to the surface. Our results suggest that monocyte/macrophage activation by metal-protein antigens can induce considerable regulation (positive or negative) of MHCII expression. However, while this may aid specific (adaptive) lymphocyte-mediated immune responses, increased MHC does not necessarily lead to lymphocyte activation, where receptor specificity for is required to initiate such a response. Activation of monocytes/macrophages in response to metal debris encompasses multiple mechanisms that are not limited to cytokine production, and also include the expression of antigen presentation along with co-stimulatory molecules. Further research is needed to determine the effects of lymphocyte recognition of specific antigenic determinants on the further activation and maturation of monocytes/macrophages.

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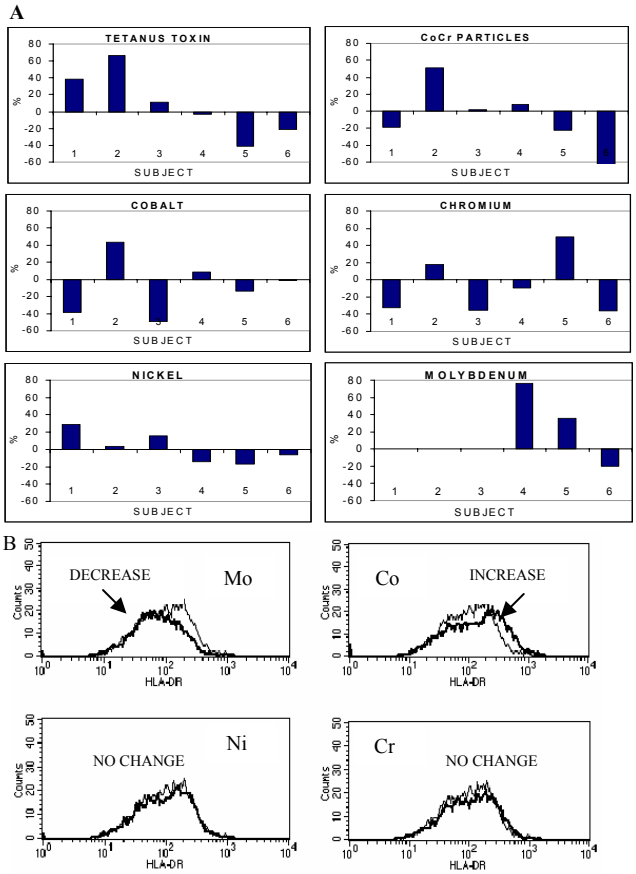

Figure 1. (A) Surface MHCII increase/decrease compared to untreated controls. (B) MFIs of MHCII (HLA-DR) Note: Metal treated (thick solid lines) untreated controls (thin lines).