MERCURY AND OTHER HEAVY-METAL INDUCED AUTOIMMUNITY.

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The heavy metals mercury and silver elicit a genetically restricted antinucleolar autoantibody (ANoA) response that targets fibrillarin, a 34 kD protein component of many small nucleolar ribonucleoprotein (snoRNP) particles. The mechanisms by which a simple chemical such as $HgCl_2$ elicits an autoantibody response that predominantly targets a single intracellular protein autoantigen remain uncertain. Possible contrbuting factors that may result in potentially immunogenic material could be direct interaction of mercury with antigen, or $HgCl_2$ mediated cell death leading to release of antigenic material. We have examined these possibilities by (a) testing the ability of mercury to interact directly with fibrillarin, or by (b) exposing mouse cell lines in vitro to $HgCl_2$ to document any changes in the cellular localization and molccular structure of fibrillarin. In the absence of 2-mercaptoethanol (2-ME) fibrillarin in isolated rat liver nuclei migrated as a well spaced doublet in SDS-PAGE. The presence of $HgCl_2$ converted fibrillarin to the faster migrating form. Other nuclear autoantigens such as lamin B, SS-B/La, DNA topoisomerase I or U1-70 kD did not exhibit such behavior. Chemical modification supported the hypothesis that the two cysteines of fibrillarin are chelating mercury to alter its migration under non-reducing conditions. Addition of 2-ME abolished this aberrant migration, with fibrillarin migrating as a single band whether $HgCl_2$ was present or not. When added to cell cultures $HgCl_2$ reduced viability of J774A.1 macrophage cell cultures in a concentration dependent manner, killing all cells

after a 3 hour exposure to 40 uM $HgCl_2$. Dead cells were characterized by condensed nuclei and cytoplasmic membrane blebs, and no longer exhibited nucleolar staining with anti-fibrillarin antibodies, although fibrillarin was present as judged by immunoblot. In the asbence of 2-ME fibrillarin from $HgCl_2$ treated cells migrated aberrantly in SDS-PAGE, as found for isolated nuclei incubated in the presence of $HgCl_2$. Addition of exogenous fibrillarin to cell lysates revealed proteolytic degradation of the protein in lysates from $HgCl_2$ treated J774A.1, but not EL 4 (T cell) or P3 (B cell) lysates or untreated control cell lysates. The protease inhibitor profile of this proteolytic activity is unlike other apoptosis related proteases of the ICE/CED-3 family. Our observations show that $HgCl_2$ interacts with fibrillarin modifying its molecular propertics and that $HgCl_2$ induced cell death is associated with changes in the cellular localization of fibrillarin. In phagocytic cells $HgCl_2$-induced cell death is associated with proteolysis of the protein. We propose that interaction of mercury with fibrillarin followed by protecolysis may allow cryptic epitopes to be generated that can be recognized by autoreactive cells in genetically susceptible ($H\text{-}2^s$) murine strains, leading to autoimmunity. Murine heavy metal-induced autoimmunity provides a model system for studying how a xenobiotic may render a nuclear self antigen immunogenic. Such observations may by of significance in understanding the immunotoxicity of xenobiotics in general.