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Evaluation of Hybridoma B9 cell line handling of Cadmium in the presence of Interleukin-6

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ABSTRACT: Induction of metallothionein synthesis in the presence or absence of Interleukin –6 (IL-6) was one of the responses examined to identify the the mechanism by which IL-6 (at high concentration) was able to reverse cadmium inhibition of B9 cell line proliferation. Cellular metallothionein was assayed by Cd-haem method. The results revealed a Cd-induced increase in cellular metallothionein synthesis. IL-6 alone was not sufficient to induce expression of metallothionein in this cell line. The Kinetics of B9 cells handling of cadmium (using radio active ^{109}Cd as tracer) was another response examined. Cells were pre-loaded with ^{109}Cd then transferred to ^{109}Cd-free medium containing IL-6. The results shows the efflux of Cd from IL-6 stimulated B9 cells which suggest that there is no functional linkage between IL-6 stimulation and cellular handling of Cd. The efflux of cadmium out of the cells is however an important observation in that many function assays used in immunotoxicology are carried out *in-vitro* after cellular exposure to the chemical *in-vivo*. In the interpretation of such data, the ability of the cells to recover as a result of cellular efflux of the chemical should always be considered. @JASEM

Interleukin-6 (IL-6) is a multifunctional cytokine involved in the regulation of immune and inflammatory responses. The functions of IL-6 include induction of haematopoietic cell proliferation this function can be mimicked *in-vitro* using hybridoma B9cell line (Lansdorp *et al*., 1986). A number of studies have reported the involvement of cytokines in metal-mediated immunomodulation. In one of such studies, it was demonstrated that mercury-induced increase in IgE and IgG2 in mouse was abrogated with specific antibody to interleukin – 4 (IL-4) (Ochel *et al*., 1991). The observation suggests that the mercury-induced increase in IgE and Ig G1, in vivo, was mediated by an increase in IL-4 production. *In vitro*, Cadmium, lead, copper and zinc (each at 10 or 100 µM) have been shown to inhibit antigen-induced interleukin-2 production by D11.10 cell line (Smith and Lawrence, 1998). The report by Scuderi, (1990) showed inhibitory effect of copper on interleukin-6 secretion by human monocytes. Kowolenko and his colleagues (1988) have demonstrated that lead acetate (50 or 100 µM) inhibited, the response of macrophage to colony stimulating factor-1 (CSF-1). Orupabo and associates (1992) have also reported the inhibitory effect of cadmium on B9 cell proliferation in response to interleukin-6. In the same study, high concentration of IL-6 reversed the inhibitory effect of cadmium. This study examines hybridoma (B9) cell line handling of Cadmium in the presence of Intreleukin-6 to identify how IL-6 (at high concentration) was able to reverse cadmium inhibition of B9 cell proliferation.

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MATERIALS AND METHODS

The IL-6 dependent B9 hybridoma cell line was propagated as established by Dr. L. A. Aarden (Lansdorp et al., 1986), Cell cultures were incubated in a humidified incubator at 5% CO_2 and 37^oC. B9 cells (5 x 10^5/well) were treated in 24 well culture plates with varying concentrations of IL-6 (0 – 1ng/ml) with or without $CdC1_2$ (`10µM) in RPMI basal medium. Cell growth was allowed to proceed for 72 hours. At the end of the incubation period, aliquot (100m1) of the cell suspension were transferred to a 96 well plate and cell proliferation measured by a bio-assay procedure using tetrazolium bromide (Mosmann, 1983). Metallothionein assay is based on the procedure as described by Easton and Toal (1982).

Measurement of ^{109}Cd Efflux: B9 cells were suspended at 5 x 105/ml in basal medium containing $CdC1_2$ (10 µM) and 1 µCi/ml ^{109}Cd in 250 ml culture flasks. After two-hour incubation period, the cells were washed three times in 10ml aliquot of normal saline before re-suspension in Cd-free medium containing IL-6 (100 pg/ml). At specified time interval, cells were harvested and efflux was determined. Cells were wash with two 3ml aliquots of normal saline then lysed with one millilitre of 1% sodium dodecyl sulphate (SDS) and then transferred to a scintillation vial for gamma counting. ^{109}Cd content at the indicated time was expressed relative to isotope present in cultures, which received the wash protocol without any intervening efflux interval. In this way, a time course of ^{109}Cd efflux was constructed by preloading sets of cultures with

radioisotopes, subjecting individual cultures to defined efflux intervals, and measuring the corresponding fractional retention of tracer ion.

RESULTS

Metallothionein level was measured in B9 cells after 72 hours in culture stimulated with varying concentrations of IL-6 in the presence or absence of $CdC1_2$ (10 µM). IL-6 alone did not enhance metallothionein synthesis in this cell line. Cadmium induced metallothionein synthesis in the cell line at the same time inhibit cell proliferation. Cell proliferation was measured to correct for cell number (Fig. 1). The relationship between IL-6 stimulation and the kinetics of Cd efflux from B9 cells was investigated. Cells were pre-loaded with ^{109}Cd under standard growth conditions followed by transfer to Cd-free medium containing IL-6. The results are presented as changes relative to isotopes present in the cell culture, which received the wash protocol without any intervening efflux interval. The result shows that by 24 hours, about 80% of the cellular Cd had diffused out of the cells (Fig. 2). This suggests that IL-6 stimulation does not control cellular handling of cadmium in this cell line

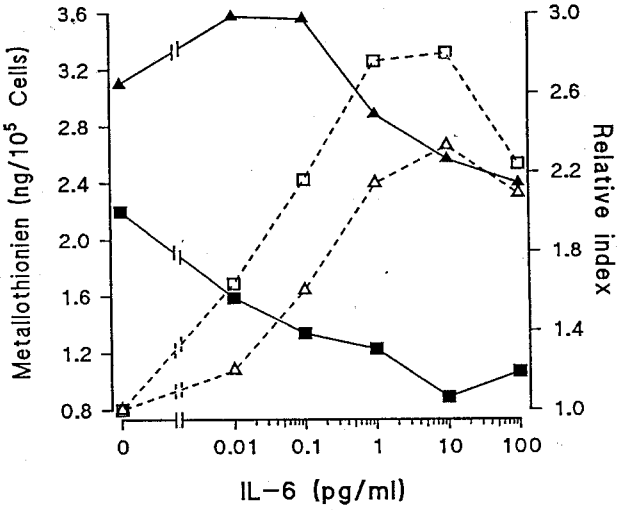

Fig 1: Effect of Il-6 And Cd On Metallothionein Induction In B9 Cells
relative proliferative index (open symbols -----) and metallothionein synthesis (solid symbols ____) in cells stimulated for 72 hours with varying il-6 in the presence (▲, Δ) or absence (■ , □) of $CdC1_2$ (10 µm). The data is representative of three separate experiments.

DISCUSSION

IL-6 alone did not induce metallothionein synthesis in this study. The observation in this study, is supported by the fact that the metallothionein mRNA analysis conducted in the report by Schroeder and Cousins, (1990) also identified zinc and glucocorticoid dependency for IL-6 stimulation of metallothionein expression. This indicate that IL-6 signal alone is not sufficient for induce expression of metallothionein. As expected, Cd induced metallothionein synthesis was demonstrated in this cell line independent of IL-6 function.

Efflux of cellular Cd from B9 cells was observed when the cells were transferred from Cd-containing medium with IL-6 to Cd-free medium. This observation is important in that many function assays used in immunotoxicology (Luster et al., 1988) are carried out *in-vitro* after cellular exposure to the chemical *in-vivo*. In the interpretation of such data, the ability of the cells to recover as a result of cellular efflux of the chemical should always be considered. . This study has demonstrated the lack of interaction between IL-6 function and cellular handling of Cadmium.

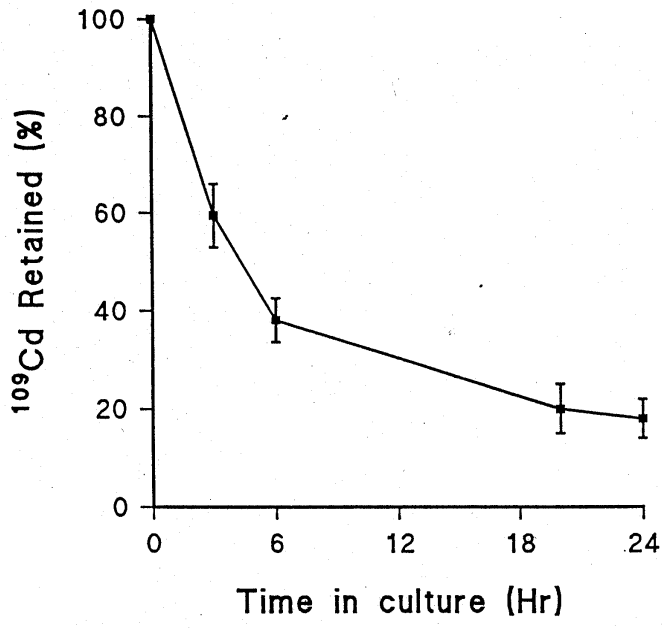

FIG 2: ^{109}Cd EFFLUX FROM IL-6 STIMULATED B9 CELLS.
Cells were pre-loaded with tracer ^{109}Cd for 2 hours followed by transfer to fresh Cd-free medium containing IL-6 (100 pg/ml).

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