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Molecular Immunological Markers for the Toxicological Investigation : Experiences from Lead-Induced Immunotoxicities

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1. Introduction

Molecular immunological methods are extensively applied to toxicological investigations. Furthermore, various immunological markers have been developed to substantiate molecular mechanisms of xenobiotics-mediated immunotoxicities. We discuss molecular immunological approach to evaluate lead (Pb)-induced immune alteration resulting in suppression of IFNγ production, and its value for establishing useful immunotoxicological markers. Observations regarding Pb-regulated expression of major histocompatibility complex or TNFα receptors will also be discussed.

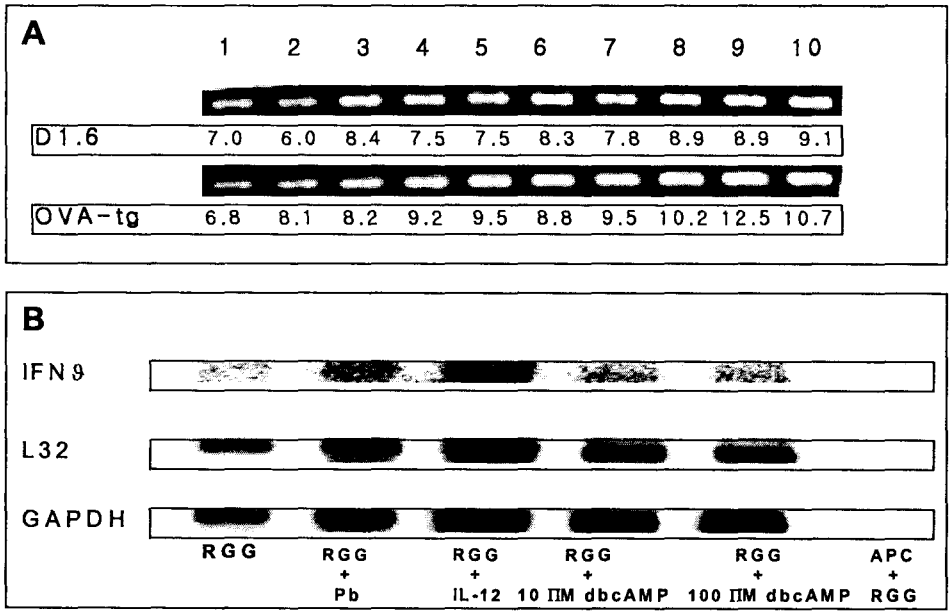
2. Materials and Methods

RT-PCR was used to detect the IFNγ mRNA in murine Th1 cells stimulated with antigen or IL-6 and IL-12 in splenocytes from Pb-exposed mice. In addition, RNase protection assay was adopted to reveal effects of lead on synthesis of IFNγ mRNA. IFNγ protein levels were quantitated in the Th1 cell culture supernatants

and the cell lysates. A possibility of Pb-induced proteosomal degradation of IFNγ protein was also tested through lactacystin treatment. To evaluate Pb's suppressive potential on IFNγ translation step, metabollic or biosynthetic labeling of the Th1 clone with [35S] methionine and subsequent immunoprecipitation using anti-mouse IFNγ mAbs.

3. Results and Discussion

1) Lead does not modulate IFNγ mRNA expression in murine Th1 clone.



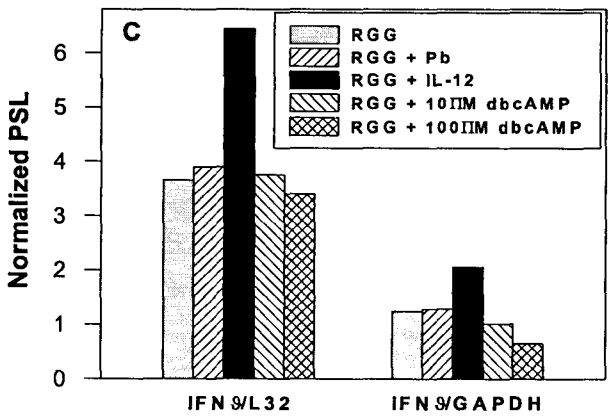


FIG. 1. Lead did not modulate IFN mRNA expression of D1.6 Th1 clone stimulated *in vitro* with antigen (RGG). For detection of IFN mRNA by RT-PCR, the D1.6 Th1 cells were stimulated with RGG for 24 hours in the presence or absence of 25 M PbCl2, 100 M dbcAMP, or 5 ng IL-12 (A top). The OVA-tg CD4+ T cells (2 x 105cells/ml) were also restimulated with OVA (0.5 mg/ml) and APC (2.5 x 106 cells/ml) for 24 hours following 6 days-*in vitro* antigenic differentiation culture done in the presence or absence of PbCl2, M dbcAMP, rIL-12, or anti-IL-4 mAb (A bottom). Numbers under the bands are relative intensity units, which are obtained through dividing each IFN density by their house keeping gene 2- microglobulin density. For detection of IFN mRNA by RPA, the D1.6 Th1 cells were stimulated with RGG for 12 hours in the presence or absence of PbCl2 (25 M), dbcAMP (10 or 100 M), or IL-12 (5 ng) (B). Normalized PSL (photostimulated luminencence) values for the RPA products were obtained through dividing each IFN PSL by the value of L32 or GADPH house keeping gene mRNA (C). The results were essentially the same in two representative separate experiments.

2) Proteosomal degradation of intracellular IFNγ is not involved with the Pb-induced inhibition of IFNγ production.

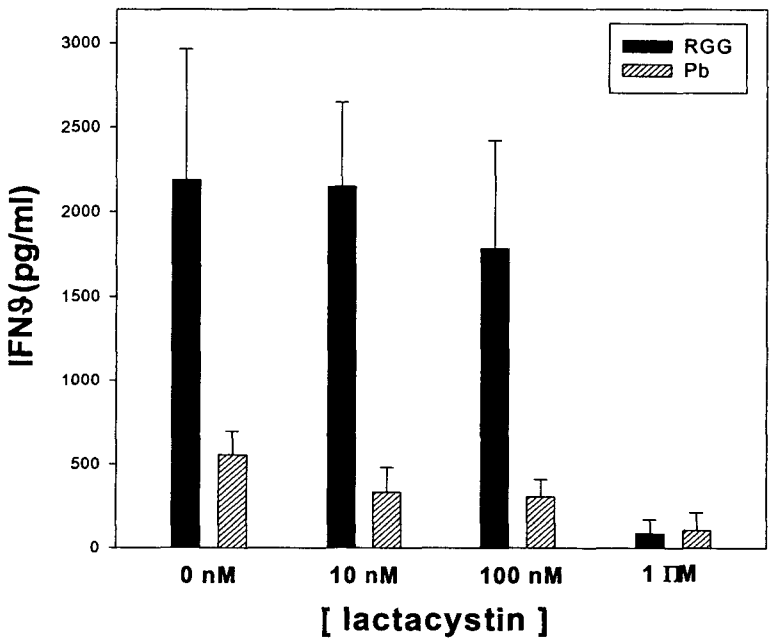


FIG. 2. Proteosomal lysis of intracellular protein was not responsible for the lowered production of IFN by Pb. D1.6 Th1 cells were treated with 10, 100 nM, and 1 M of lactacystin followed by stimulation with the antigen, RGG. Culture supernatants collected at 36 h were used for IFN quantitation. The results are expressed as the means (SEM) of three separate experiments. The ($p<0.05$) indicates significant difference from the antigen control.

3) Pb reduces the biosynthesis of IFNγ protein.

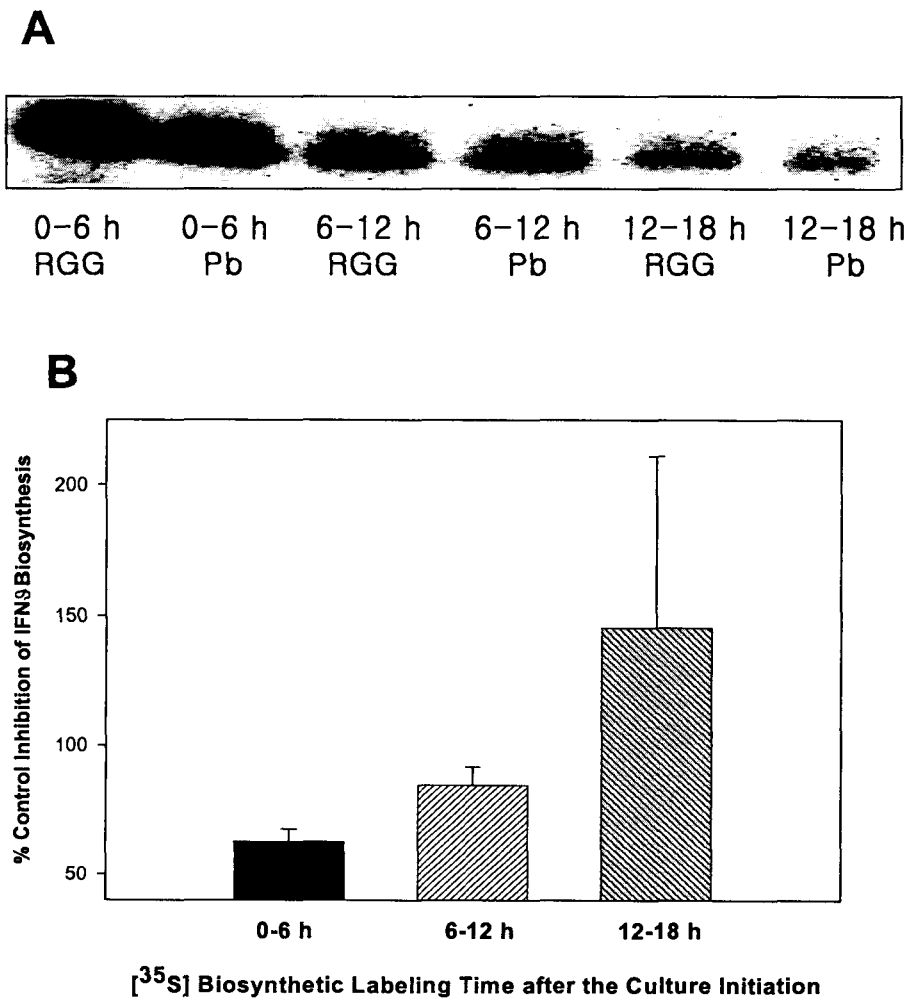


FIG. 3. Pb exerts a downregulatory role for the biosynthesis of IFN in D1.6 Th1 clone. Resting D1.6 Th1 cells were pulsed using the [35S] Protein Labeling Mix for 6 hours at 6 hour intervals after initiating stimulation with antigen and APC in the presence or absence of 25 M PbCl2,and the chase with non-radiolabeled amino acids was followed by the end of 18h- stimulation. Culture supernatants were collected at the end of stimulation and were immunoprecipitated, fractionated by SDS-PAGE. Levels of biosynthetically labeled IFN were assessed by phosphorimaging analysis (A) and calculated by dividing PSL of Pb-treated cells by that of the antigen control. The results are expressed as the means (SEM) of two separate experiments (B). The ($p<0.05$) or ($p<0.01$) indicates significant differences significant difference from the antigen control.

4) Summary and conclusion

Lead (Pb) is known to preferentially suppress activation and development of type-1 helper T cells, whereas enhance the development of type-2 helper T cells and its activities. Inhibition of IFNγ production was demonstrated in vitro from Th1 clone, splenic T cells exposed to Pb. Therein, we investigated intracellular mechanisms leading to the Pb-induced downregulation of IFN production. Expression of IFN mRNA by a Th1 clone stimulated with antigen was examined. No Pb effects on IFN mRNA expression was shown. IFN levels in the cell lysates were determined after antigenic-activation of the Th1 clone in the presence or absence of Pb. Pb supplementation resulted in significantly lowed IFNγ level in the cell lysates in comparison with that of the antigen control, suggesting that Pb-mediated cytoplasmic accumulation of IFNγ may not be undergone for the decreased IFNγ secretion. Pb-driven potentiation of IFNγ protein degradation was evaluated through comparing IFNγ levels in the Th1 cells pre-treated with lactacystin, the most effective blocking agent on proteosomal lysis, with those in the untreated cells. IFNγ production was remained suppressed regardless of lactacystin pre-treatment, indicating no aberrant triggering of IFNγ protein proteolysis by Pb. The influence of Pb on IFN biosynthesis was investigated using 35S-incorporation pulse/chase experiment. IFNγ synthesis in the Pb-treated Th1 cells was significantly reduced at the 0-6 h and 6-12 h pulse period, but not at the 12-18h compared with that of the antigen control. ur results suggests that the

reduced secretion of IFNγ does not relate to inhibition of transcription, but rather to defects at post-transcriptional processes prior to secretion of IFN production.