2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN (TCDD) EXPOSURE INCREASES A NOVEL CELL POPULATION IN THE SPLEEN OF P815-TUMOR-INJECTED MICE: CHARACTERIZATION OF MAC-1$^+$GR-1$^+$ CELLS

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Introduction

TCDD, a highly toxic environmental contaminant and prototypic ligand for the cytosolic Ah receptor, suppresses both cell-mediated and humoral immune responses. Despite considerable investigation, the mechanism by which TCDD suppresses immune responses remains unknown. Our laboratory has reported that, in C57Bl/6 mice, exposure to TCDD induces a dose-dependent suppression of cytotoxic T lymphocyte (CTL) activity and allo-specific antibody responses following the injection of allogeneic P815 tumor cells[1]. In addition, we have reported the presence of a large number of Mac-1$^+$ cells in the spleens of TCDD-treated mice at the same time that TCDD suppresses CTL development in P815-injected mice[2].

Further studies in our laboratory have shown that these Mac-1$^+$ cells are present in the blood as well as in the spleen of P815-injected mice and that they co-express Gr-1, a myeloid antigen which is a marker of granulocyte differentiation and maturation. Since Bronte *et al.*[3] showed that Mac-1$^+$Gr-1$^+$ cells inhibited the development of an immune response in mice infected with vaccinia virus, we further examined the effect of TCDD exposure on the Mac-1$^+$Gr-1$^+$ population in P815-allografted mice and examined their potential immunomodulatory function.

Materials and Methods

Male C57Bl/6 mice were treated with vehicle or TCDD (15 μg/kg) one day prior to the injection of allogeneic P815 tumor cells. At various times after P815 injection, spleen cells and peripheral blood cells were stained for the expression of various cell surface markers. Cells from both compartments were characterized by immunohistochemistry and morphological examination. Cytolytic activity toward P815 or YAC-1 cells was measured in a standard chromium release assay (CRA) following in vivo or in vitro stimulation. In order to assess their potential immunomodulatory role, Mac-1$^+$Gr-1$^+$ cells were enriched from the spleen by negative panning using mAbs to CD4, CD8, and mouse IgG and from the blood by Ficoll-gradient centrifugation. These enriched Mac-1$^+$Gr-1$^+$ cells were then co-cultured with naive spleen cells to determine their effect in a mixed lymphocyte response to allogeneic P815 tumor cells.

Results and Discussion

Morphological analysis identified Mac-1^+Gr-1^+ cells as neutrophils in the blood and as neutrophils and immature granulocytes in the spleen of vehicle-treated mice. Immunohistochemical analysis showed that these cells were localized in the red pulp in the spleen. Flow cytometric analysis showed that, in addition to Mac-1 and Gr-1, these cells expressed ICAM-1, LFA-1, MHC Class I, CD40, F4/80 and B7-2 molecules.

Exposure to TCDD augmented the induction of Mac-1^+Gr-1^+ cells in the spleen and blood but these cells were not morphologically different from vehicle-treated mice. However, their expression of ICAM-1 and B7-2 was down regulated. CD62L was completely shed, indicating that these cells were highly activated.

In vehicle-treated mice, Mac-1^+Gr-1^+ cells exhibited cytolytic activity against YAC-1 tumor cells and produced superoxide upon stimulation with PMA. In contrast, Mac-1^+Gr-1^+ cells from TCDD-treated mice exhibited no cytolytic activity to YAC-1 cells, indicating that TCDD treatment disrupted the cytolytic function of these Mac-1^+Gr-1^+ cells. However, these cells produced superoxide at higher levels than vehicle-treated mice, indicating that these cells were activated.

Mac-1^+Gr-1^+ cells from TCDD-treated mice suppressed the *in vitro* generation of CTL activity when naive spleen cells were incubated with mitomycin-C treated P815 tumor cells. However, this suppression required close cell-cell contact.

Taken together, these data show that TCDD treatment results in an enhanced inflammatory response, as reflected by the large increase in Mac-1^+Gr-1^+ cells in the spleen and blood. These cells appear to be highly activated as determined by the loss of CD62L expression and their production of superoxide. However, their ability to directly lyse tumor cells was completely abrograted. In addition, they appeared to suppress the *in vitro* generation of allospecific CTL effector cells by a cell-cell dependent mechanism.

References

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