EFFECT OF 2,3,7,8-TETRACHLORODIBENZO-*P*-DIOXIN (TCDD) ON B CELL DIFFERENTIATION IN MOUSE PRE-B COLONIZATION MODEL REGULATED BY ARTIFICIALLY INTRODUCED HUMAN IL-3 RECEPTORS

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Abstract

A model for pre-B stem-cell colonization has been established by transgenic technology by introducing the human IL-3 receptor gene. Pre-B stem cells from the bone marrow undergo continuous proliferation only when human IL-3 is present, and differentiate when IL-7 is added to the culture. Using the model, the effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on B cell differentiation after colony replating was evaluated, and it was found that TCDD induced a decrease in the number of pre-B stem cells in a dose-dependent manner, specifically in a fraction of the differentiated pre-B stem cells, implying lower AhR expression in immature pre-B stem cells.

Introduction

The effects of TCDD exposure on hemopoiesis have been extensively investigated after suppressive changes in the bone marrow as well as immunological parameters, including CFU-GM and other progenitors, were reported for the first time by Luster et al. in the early 80's (1). Thereafter, specific attention was given to the suppression of B cell and B lymphopoiesis (2,3), and these alterations were later found to be medi-ated by the aryl hydrocarbon receptors, AhRs (4,5). Interestingly, we found that the down-regulation of AhR expression attenuated myelosuppression in thioredoxin (Trx/ADF) over-expressing mice observed in a hemopoietic colonization assay, which clearly revealed the linkage of AhR signals to the antioxidant cascade that is induced by reactive oxygen species (ROS) after TCDD exposure (6-9).

Previously, we established a series of transgenic mice carrying one of the subunits of human IL-3 receptors (h-IL-3Rs), alpha and beta (10, Figure 1), in which murine *de novo* IL-3R, not only in the bone marrow cells but also in other tissues, does not respond to human IL-3, whereas the h-IL-3R introduced to the mice responds to human IL-3. Consequently, hemopoietic colonization assay on the bone marrow cells provides a unique 'stem cell' assay system originating from pre-B cells, that solely proliferate in the presence of human IL-3 without differ-entiation, but, undergo differentiation in the presence of IL-7. This pre-B stem-cell colony may be a good tool for evaluating the effect of exposure to environmental chemicals on cell proliferation and/or differentiation.

In this study, effect of TCDD on pre-B stem cell colonization was investigated and it was found that specific suppression of B cell differentiation is induced by in vitro exposure to TCDD. Our pre-B stem cell colonization assay is a potential tool for evaluating chemicals such as halogenated environmental organic pollutants and POPs, which potentially modify the stem cell propensity in differentiation.

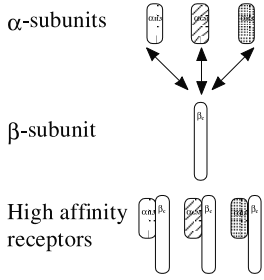

Figure 1. High affinity human interleukin receptors for IL-3, GM-CSF, and IL-5. Sharing of signal-transducing subunit, common beta, with each specific alpha subunit.

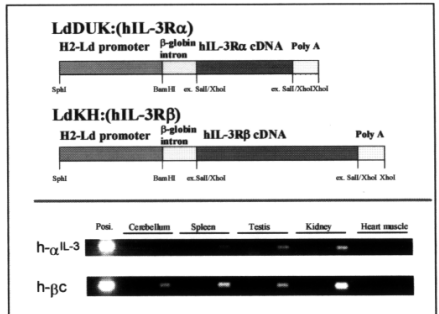

Figure 2. Top, Gene construction of transgene for h-IL-3R alpha (DUK) and beta (KH), and Bottom, the expression profile in the major organs.

Materials and Methods

h-IL-3R transgenic mic

High affinity IL-3Rs are composed of alpha-beta hetero-dimers (Figure 1). In this study, the alpha and beta subunits of h-IL-3Rs were separately introduced into fertilized eggs, and then the eggs were injected into pseudo-pregnant mice. An MHC-L^d promoter was used for ubiquitous expression (Figure 2). Mice carrying both subunits of h-IL-3Rs were produced by mating with hemizygous transgenic mice with alpha or beta subunits. Consequently, the developed mice showed neither behavioral nor hematological alteration, except an overgrowth under exposure of human IL-3 in the bone marrow culture.

Hemopoietic in vitro colony assay

Bone marrow cells removed from h-IL-3R mice or their wild type counterparts were re-suspended in a-MEM, plated into 3cm petri dishes at 2 x 10^4 cells/ well, and cultured for 9 days in 0.8% semi-solid methyl-cellulose culture with or without 10 ng/mL human IL-3, supplemented with 30 % fetal bovine serum, High Clone^R, 1 % bovine serum albumin, and 10^{-4}M 2ME. Bone marrow cells from mice carrying h-IL-3Rs produced an average of 41.7 colonies per 2x10^4 cells plated when human IL-3 was added into the culture; in contrast, no colonies were observed when human IL-3 was not added, or when the bone marrow cells were from non-transgenic wild type mice. Furthermore, there was absolutely no difference in the number of colonies developed between bone marrow culture of h-IL-3-Rs and that of wild type mice when murine IL-3 was added.

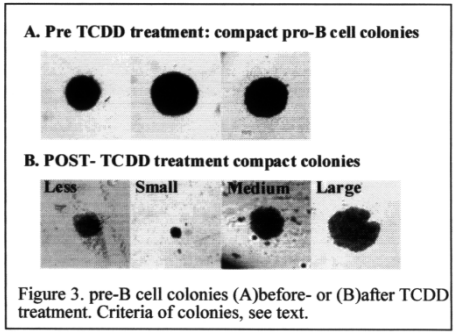

Figure 3. pre-B cell colonies (A)before- or (B)after TCDD treatment. Criteria of colonies, see text.

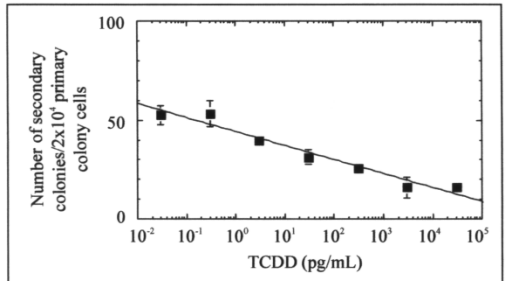

Figure 4. Total number of colonies combined with each type of colony groups vs. graded dose of TCDDs treated.

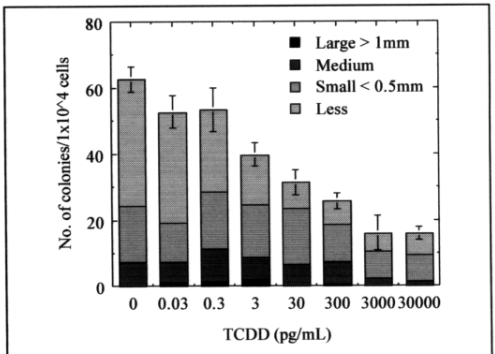

Figure 5. Effect of a graded dose of TCDD on each type of pre-B stem cell colonies.

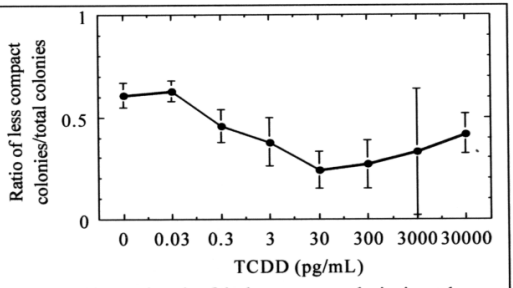

Figure 6. Changes in ratio of the less compact colonies in total number of the compact colonies counted along the dose of TCDD.

B cell colonization

In Figure 3A, among the colonies developed in the presence of human IL-3, bone marrow cells from h-IL-3Rs produced nearly 25 % discrete and compact colonies (Figure 3A). Using a cell sorter, it was found that the developed compact colonies were $CD19^+$, $CD43^+$, surface IgM^{low}, and $B220^+$, *i.e.*, they originated from pre B cells. Gr-1 and Thy-1 were negative. On the 9th day of culture, the compact colonies were lifted by a micropipette and replated into secondary wells with the same medium composition as that used in the primary methylcellulose culture. More than 90 % of the compact colonies were observed from the replating culture, and therefore this culture system was called the "pre B stem-cell model". On the other hand, when 10 ng/mL murine IL-7 was added to the replating culture, the system became a tool for evaluating potentially hazardous compounds for B-cell differentiation and proliferation.

TCDD exposure

To the above culture system was added a graded dose of the test compound, TCDD, of 0.3, 3.0, or 30.0 pg/mL, or mock media. On the 12th day of culture, the number and shape of colonies were counted and evaluated with an inverted microscope.

Results and Discussion

Decrease in number of replating colonies

After TCDD exposure, almost all of the colonies that developed were compact colonies. They were lifted by a micropipette and their shape and size characteristics evaluated according to the following

criteria. a large compact colony, more than 1 mm in diameter; a medium- sized compact colony, between 1mm and 0.5 mm in diameter; and a small compact colony, less than 0.5 mm in diameter (Figure 3B). In Figure 4, the total number of colonies, shown on the ordinate, decreases with a graded dose of TCDD shown on the abscissa, implying that the number of pre-B progenitor cells was decreased by TCDD in a dose-dependent manner.

Less compact pre-B cell colonies are more susceptible to TCDD toxicity

The above result implies that the less compact colonies are more sensitive to TCDD toxicity, as shown in Figure 5. In this Figure, the percentages of less compact colonies for the case of 0.03 pg/mL TCDD and that of TCDD-free control were 63% and 61%, respectively, whereas the percentage less compact colonies for the case of 3000pg/mL TCDD was low as 33%. These results imply that the less compact colonies derived from the pre-B stem cells were more susceptible to TCDD toxicity. These results are in good agreement with the current report of Thurmond, et al. (**11**).

Ratio of less compact colonies to total colonies decreases with increasing dose of TCDD

From the number of each type of compact colonies counted above in Figure 5, the ratios of less compact colonies to the total colonies are plotted vs. the dose of TCDD from 0.03 through 3 x 10^4 pg/mL (Figure 6). The ratios of the less compact colonies started to decrease from 61 % (TCDD-free control) to 30% (TCDD at 30 pg/mL) and then reached a plateau at doses higher than 30 pg/mL.

Conclusion

The difference in susceptibility to TCDD toxicity between relatively differentiated pre-B progenitors and other immature pre-B progenitors might be due, in part, to the possible difference in expression of AhR along with the differentiation in the B-cell lineages.

Acknowledment

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