Thymus stroma mediated changes in fetal thymocyte differentiation by 3,3',4,4'-tetrachlorobiphenyl

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Polychlorinated hydrocarbons such as biphenyls and dioxins interfere with cellular processes by gene induction via ligand activated binding of the cytosolic Ah-receptor to specific DNA elements. The thymus is a target organ for these processes as the Ah-receptor is abundant in this tissue, and immunosuppression a hallmark of polychlorinated hydrocarbon toxicity in all species. The thymus is the major anatomical site for the development of mature, immunocompetent T-cells, requiring proper interactions between stroma and thymocytes: stroma cells present self-peptides in the context of the major histocompatibility complex to thymocytes and provide developmental signals via adhesion molecules and cytokines. Interference of xenobiotic substances could result in changes of cellularity, skewed subpopulations, impaired functionality of the thymocytes/T-cells, or combinations of either.

We analysed the effects of 3,3',4,4'-tetrachlorobiphenyl on the development of fetal thymocytes ex vivo in `Fetal Thymus Organ Cultures' exposed to TCB. Exposure of fetal thymi for 7 days, starting at day 15 of gestation, resulted in a dose-dependent decrease in thymocyte number and frequency changes of thymocyte subpopulations. Differentiation stages of thymocytes as defined by the expression of cell surface markers CD4 and CD8 were skewed by TCB. Maturation, i.e. appearance of the various CD4/CD8 bearing subpopulations ($CD4^-CD8^-$, $CD4^+CD8^+$, $CD4^-CD8^+$, $CD4^+CD8^-$) was accelerated by TCB, and after 7 days of culture, $CD4^+CD8^+$ cells were significantly decreased, whereas $CD4^-CD8^+$ `single positive' cells were abundant. These $CD8^+$ cells are mature as judged by expression of e.g. the α/ß-T-cell receptor and their lack of precursor activity: $CD4^-CD8^+$ cells isolated from 7 day old organ cultures did not show any differentiation in recultivation experiments, irrespective of TCB treatment (see Table 1). Furthermore, the percentage of CD44 expressing $CD4^-CD8^-$ thymocytes increased significantly by TCB treatment. CD44 is important for lymphocyte-epithelial interactions and we suggest that the increase in CD44 bearing cells interferes with proper migration of thymocytes through the stroma. This is also demonstrated by the emigration of thymocytes from the lobes: upon TCB treatment $CD4^+CD8^+$ and $CD4^-CD8^+$ cells are preferentially retained, and all emigrated thymocyte subpopulations have a bias towards CD44 expression. In order to determine the role of the thymus stroma for the observed changes in thymocyte subpopulation patterns, we depleted fetal thymus lobes from thymocytes by treatment with desoxyguanosine.

The empty lobes were then recultivated with $CD4^-CD8^-$ cells (i.e. the most immature thymocytes subpopulation), isolated from gestation day 15 embryos, or from 7 day old thymus organ cultures. Either the lobes or the thymocytes used for recultivation were exposed to TCB, and subsequently the differentiation potential of the thymocytes was analysed. As shown in Table 1, thymocyte differentiation of recultivated thymocytes is not supported by lobes (i.e. thymus stroma) that had been treated with TCB. In contrast, pre-treatment of double negative thymocytes with TCB did not impair their capacity to give rise to all more mature thymocyte subpopulations. This is direct evidence that the thymus stroma/epithelium and not thymocytes themselves is the target of TCB action.

TABLE 1: Differentiation potential[a] of thymocytes in organ culture

			Thymus lobes	
			control	3.3µM TCB
$CD4^-CD8^-$	from	control	yes[b]	no
		3.3µM TCB	yes	no
$CD4^-CD8^+$	from	control	no	no
		3.3µM TCB	no	ND

[a] thymocyte depleted fetal thymus lobes were treated for 5 days with 3.3µM TCB and recultivated with the indicated thymocyte subpopulation for another 7 days. Thymocytes were isolated, stained for CD4 and CD8 expression and the percentage of CD4/CD8 expressing cells determined.

[b] 'Yes' and 'no' indicate, whether $CD4^+CD8^+$, $CD4^-CD8^+$, and $CD4^+CD8^-$ cells were generated.

ND = not done