TRANSGENIC MICE EXPRESSING A CONSTITUTIVELY ACTIVE MUTANT OF THE DIOXIN RECEPTOR

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Transgenic animals are often used in biomedical research as powerful model systems to study the role of individual genes in the intact animal. One approach involves targeted disruption of genes in order to examine the resulting phenotype of animals lacking a functional protein. This approach often gives essential information about the endogenous role of a gene and its gene product. Two independent research groups have recently utilised this approach (*1* and *2*) to create mice lacking a functional Ah-receptor, the receptor that is believed to mediate most, if not all of the toxic effects of TCDD. These mutant mice have somewhat conflicting phenotypes but both mutants show decreased liver weights and fatty metamorphosis of hepatocytes. A more long-term histopathological study also revealed cardiomyopathy, vascular hypertrophy in the liver and uterus as well as gastric hyperplasia (*3*). We have chosen to study the Ah-receptor by an alternate approach that is more relevant for toxicological research. We have created transgenic mice expressing a constitutively active form of the Ah-receptor, thereby mimicking a state in the non-treated animal where ligands such as TCDD or planar PCBs are always present. These animals will hopefully be important tools for mechanistic studies and will probably clarify the role of the activated receptor in dioxin toxicity.

The dioxin receptor

Dioxins and planar PCBs bind to an intracellular cytosolic receptor protein, present in all vertebrates so far examined (*4*), known as the Aryl hydrocarbon receptor (AhR). Very recently, an AhR homolog was discovered in the nematode *Caenorhabditis elegans* (*5*), and analysis of the Drosophila *spineless* (*ss*) mutant responsible for transformation of antenna and leg was identified to encode a protein with high identity to the mammalian AhR (*6*). The Ah receptor is a member of the bHLH/PAS class transcription factors. Members of this class of proteins share a basic helix-loop-helix motif important for both DNA binding and dimerisation. The member proteins also share a PAS domain which has been shown to be important for protein-protein interactions and, in the case of the DR, ligand binding activity. Novel members of this bHLH/PAS family of transcription factors are rapidly being discovered and are found to function as, for example central regulators in response to hypoxic conditions (Hif-1α,*7*), development of the central nervous system in *Drosophila* (sim, reviewed in *8*), circadian rhythm in mouse (Clock, *9*) and *Drosophila* (Per, *10*).

In absence of ligand, the AhR exists as a heterodimeric complex with the molecular chaperone Hsp90, the role of which is to maintain the receptor in a ligand-responsive state. Ligand-binding results in release of Hsp90, dimerisation with the bHLH/PAS partner protein Arnt (AhR nuclear translocator) and translocation of the activated receptor to the nucleus. The AhR/Arnt heterodimer can then bind specific regulatory elements, known as Xenobiotic Response Elements (XRE), and activate transcription of a number of genes such as cytochrome P4501A1 (*Cyp1a1*). Our transgenic mice express a mutated receptor that bypasses this ligand dependency for activation and is activated and functions as if ligand was bound.

Receptor-mediated toxicity

It is generally believed that most, if not all the toxic effects of dioxins and planar PCBs are mediated by the ligand-bound, activated dioxin receptor. However, there is a remarkable difference in toxic sensitivity to ligands between species (1000 fold) and even between inbred strains within a species (*11*). This differential sensitivity segregates with alleles for a high and low affinity receptor (*12*), supporting the role of the receptor in dioxin toxicity. Further evidence has come from the generation of DR-deficient mice that are resistant to the acute toxicity of very high doses of the most potent dioxin TCDD (*13*). Most of the risk assessment of dioxins and planar PCBs is based on the mechanistic concept of binding of the congeners to the receptor with subsequent activation, leading to regulation of gene transcription. The transcriptional up-regulation of the *Cyp1a1* gene has been used as a well-studied and sensitive marker for an ligand-bound, activated Ah-receptor. The transcriptional induction of *Cyp1a1* mRNA can be measured by Northern blot and RT-PCR. The increased enzymatic activity of the translated Cyp1a1 can be measured with the AHH or EROD assay. Several of these different assays have been used extensively both in vivo and in vitro to assess the potency of several congeners, often compared to TCDD. We have used several of these assays to test whether the mutated Ah-receptor expressed in our mice functions as a constitutively active, ligand-independent regulator of *Cyp1a1* transcription.

The transgenic mice

Our transgenic mice have been bred to homozygosity and the tissue-specific expression of the transgene has been analysed. We wanted to get high ectopic expression of the transgene in the immune system by using an enhancer/promoter construct shown to promote strong expression in B- and T-lymphocytes (*14*) To this end we have shown, by both RT-PCR and Northern blot analysis, successful expression of the transgene mRNA in both thymus and spleen. We can also detect expression of transgene mRNA in several other tissues which have been analysed by RT-PCR. The expressed mutant receptor appears to be functional since induced expression of *Cyp1a1* mRNA can be observed in a ligand-independent manner detected by both Northern blot and RT-PCR. An increased EROD activity has also been seen in several tissues. Further studies are needed to get a more complete picture of transgene expression and its ability to regulate gene transcription in a ligand-independent manner. However, we belive that these mice will turn out to be very useful in mechanistic studies of dioxin toxicity and also to verify the mechanistic concept on which most risk assessment of dioxins and planar PCBs rely.

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