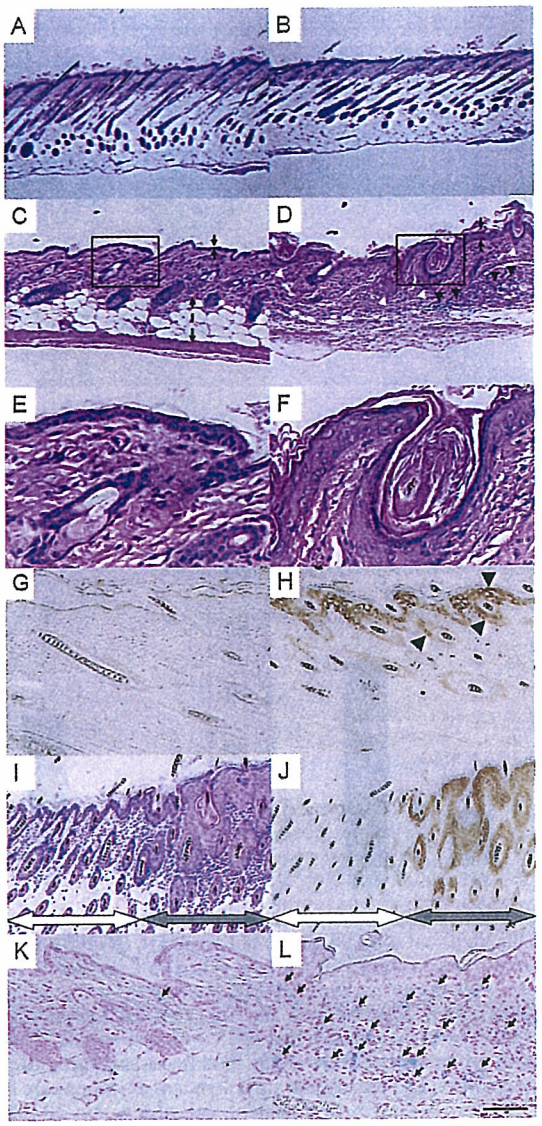

FIG. 3. Histological examination of the dorsal skin of AhR-CA mice. (A to F) Skin specimens from Line 239 AhR-CA transgenic mice (B, D, and F) and from wild-type mice (A, C, and E) were stained with hematoxylin and eosin. The samples were prepared at the age of 1 week (A and B) or 4 weeks (C to F). The black arrows (C and D) indicate the widths of the epidermal layers, and the dashed double arrowheads (C) indicate the width of the subcutaneous fat tissue. Black arrowheads (D) indicate cell infiltration, and white arrowheads (D) indicate dilated hair follicles filled with keratinized cell masses. Boxed areas in panels C and D are shown at a higher magnification in panels E and F, respectively. (G and H) Immunohistochemical detection of CYP1A1 expression. A 10-day-old Line 239 mouse (H) and its wild-type littermate (G) were examined. Intense brown signals can be seen in the epidermal and follicular keratinocytes (arrowheads) of the transgenic mouse, whereas no apparent signals can be detected in the control sample. (I and J) Serial sections prepared from the skin of a Line 234 adult female mouse. One was stained with hematoxylin and eosin (I), and the other was exposed to anti-CYP1A1 antibody (J). The left-hand halves of the micrographs (indicated by white double arrowheads) show the normal state of the skin, while the right-hand halves (indicated by gray double arrowheads) show inflammatory changes. (K and L) Alcian blue staining of the skin specimens from the 4-week-old mice of Line 239 (L) and wild-type littermate (K). Black arrows (K and L) indicate mast cells that have infiltrated the dermis. Scale bar: 40 μm (A and B), 16 μm (C, D, K, and L), 8 μm (G to J), 4 μm (E and F).

not shown), skin disorders accompanied by itching and scratching developed postnatally in Line 239 mice, suggesting that postnatal epidermal development and/or mechanical stimuli may be required in addition for completion of the skin lesion pathogenesis.

Epidermal and dermal pathological changes observed in AhR-CA transgenic mice. To examine the skin of symptomatic Line 239 mice in more detail, histological analysis was performed. Consistent with the macroscopic observation, there was no apparent abnormality during the first week after birth (Fig. 3A and B). However, prominent acanthosis and hyperkeratinization developed by 4 weeks of age (Fig. 3D and F); most hair follicles were dilated and filled with layers of keratinized cells (6, 37). The dermis was severely infiltrated with lymphocytes and polymorphonuclear cells, and the subcutaneous fat tissue had mostly disappeared (Fig. 3D and F).

Although the macroscopic skin lesions observed in Line 234 were milder than in Line 239, both lines shared similar histopathological changes. Male Line 234 mice, with the highest transgene expression (see Fig. 1B), developed the pathological state by 2 weeks of age, which was slightly earlier than the onset of Line 239 mice (data not shown). Further exacerbation could not be observed in male Line 234 mice since the mice die before weaning due to a probable insertion mutagenesis or disruption of an essential gene on the X chromosome by the transgene. Intriguingly, adult female Line 234 mice displayed chimeric patterns in their skin lesions, which made the dermatitis macroscopically less apparent. Both normal and inflammatory parts resided side by side (Fig. 3I), and this pattern was quite consistent with that of the target gene expression (Fig. 3J). This observation may be best explained by lionization of the transgene integrated into X chromosome. We can assume that, in the normal part of skin, the AhR-CA transgene was inactivated due to the lionization of the transgene-harboring X chromosome, whereas, in the inflammatory part of the skin, the expression of the transgene persisted due to the transgene-harboring X chromosome escaping from the lionization. None of our low AhR-CA transgene expressor lines showed any such skin abnormalities (data not shown). These results thus indicate that the severity of the skin disorders correlates well with the expression levels of the AhR-CA transgene.

We then monitored the activity of AhR-CA protein in the skin through detecting expression of its target gene, CYP1A1. When CYP1A1 expression in the transgenic mice was examined immunohistochemically, intense signals were detected specifically in epidermal keratinocytes, an observation consistent in Line 239 mice (Fig. 3H) and male Line 234 mice at 10 days after birth (data not shown). As expected, female Line 234 mice displayed a chimeric staining pattern (Fig. 3J). A patch of epidermis with intense CYP1A1 expression displayed acanthotic proliferation and hyperkeratinization, and the underlying dermis was infiltrated with inflammatory cells (Fig. 3I and J). However, such skin alterations were not observed in the portions without a detectable CYP1A1 expression. These results clearly indicate that the AhR pathway is activated specifically in keratinocytes and that this keratinocyte-specific AhR activation causes the observed inflammatory skin disorders.

Alcian blue staining of the skin sections revealed that a large number of mast cells had accumulated in the dermis in Line 239 (Fig. 3L). This was also evident in Line 234 males at 10 days after

TABLE 1. Genes increased in the skin of AhR-CA mice

Gene category	Gene subcategory	Accession no.	Gene	Fold change
Detoxifying enzymes	Detoxifying enzymes and genes	NM009644	Aryl-hydrocarbon receptor repressor	16.6
		NM009992	Cytochrome P450, 1a1	44.2
		NM009994	Cytochrome P450, 1b1	4.0
		NM007825	Cytochrome P450, 7b1	3.7
		AV158882	NAD(P)H dehydrogenase, quinone 1	3.8
		NM010357	Glutathione *S*-transferase, alpha 4	8.2
		NM008184	Glutathione *S*-transferase, mu 6	2.0
		NM008161	Glutathione peroxidase 3	2.7
Interleukins, chemokines, and receptors	Interleukins and their receptors	AF000304	IL-4 receptor, alpha	15.1
		L20048	IL-2 receptor, gamma chain	8.0
		D13695	IL-1 beta	6.0
		NM008360	IL-18	2.2
	Chemokines	BC002073	Chemokine (C-C motif) ligand 6	18.5
		NM009141	Chemokine (C-X-C motif) ligand 5	22.2
		AF099052	Chemokine (C-C motif) ligand 20	14.9
		NM008176	Chemokine (C-X-C motif) ligand 1	8.9
		NM011888	Chemokine (C-C motif) ligand 19	8.4
		NM021443	Chemokine (C-C motif) ligand 8	6.3
Immunological proteins	Fc receptors	NM010185	Fc receptor, IgE, high-affinity I, Gamma polypeptide	23.9
		BM224327	Fc receptor, IgG, low-affinity IIb	6.8
	Antimicrobial peptide	NM019728	Defensin beta 4	38.3
		NM013756	Defensin beta 3	11.0
Structure proteins	Keratins	NM010669	Keratin 6b	35.1
		NM008470	Keratin 16	7.8
		NM008473	Keratin 1	6.1
Proteases and inhibitors	Proteases	NM008572	Mast cell protease 8	11.1
		NM008571	Mast cell protease 2	2.8
		NM008607	Matrix metalloproteinase 13	34.8
		AV375008	Matrix metalloproteinase 19	9.1
		NM013599	Matrix metalloproteinase 9	3.3

birth (data not shown) but less evident in Line 234 females. Since mast cells are considered to be a critical factor inducing skin itching, we can deduce that the skin lesions are intimately associated with the itching.

Inflammation-related genes are increased in AhR-CA mice. To further clarify the molecular basis of the skin lesions, we performed a DNA microarray analysis with skin RNA samples from 10-day-old Line 239 mice, an age representing initiation of the skin disorders (Table 1 and Fig. 4). This early stage was chosen because we were curious about how the skin lesions were initiated. Total RNAs from five Line 239 mice and four wild-type littermates were pooled as AhR-CA RNA and wild-type RNA, respectively, and these two samples were compared. Among 309 genes that were increased >4-fold in the transgenic mice compared to wild-type mice, 7% were involved in detoxification, while, surprisingly, nearly a quarter were inflammation-related genes, including interleukins/chemokines and their receptors, immunological proteins, and proteases and their inhibitors (Fig. 4A). We looked at genes with an expression either upregulated or downregulated by at least twofold and found that the ratios of the upregulated genes were higher in the categories of the inflammation-related genes (Fig. 4B).

We then selected representative genes from the microarray analysis and examined their expression levels by RT-PCR analysis with 10-day-old skin RNAs (Fig. 4C). All of the genes, except for heme oxygenase-1 (HO-1), were consistently increased in Line 239 and male Line 234 mice and slightly increased in female Line 234 mice. For instance, the detoxifying enzymes CYP1B1, NAD(P)H:quinone oxidoreductase 1, alcohol dehydrogenase, and AhR repressor were higher in the transgenic mice compared to wild-type levels. As for the inflammation-related genes, keratins 1, 6, and 16 (K1, K6, and K16), CCL20, and IL-18 were dramatically increased in Line 239 and male Line 234 mice, reflecting the proinflammatory state of the skin of these transgenic mice.

We found that IL-18 and CCL20 were also highly expressed in the fetal skin of AhR-CA mice soon after the start of transgene expression (data not shown). In addition, considering that the upstream regions of the *IL-18* and *CCL20* genes contain multiple AhR binding sites (xenobiotic response elements [XRE]), *IL-18* and *CCL20* could be the primary genes activated by AhR-CA in keratinocytes to trigger the inflammatory responses.

An intriguing observation was that, HO-1, considered a sensitive marker of the oxidative stress generated by exposure to PAHs (20), was not induced in the AhR-CA transgenic mice, suggesting that contribution of reactive oxygen species to the development of the skin lesions might be marginal.

The cell-mediated immune response is predominant in AhR-CA transgenic mice. We then examined characteristics of fully developed skin inflammation observed in AhR-CA trans-

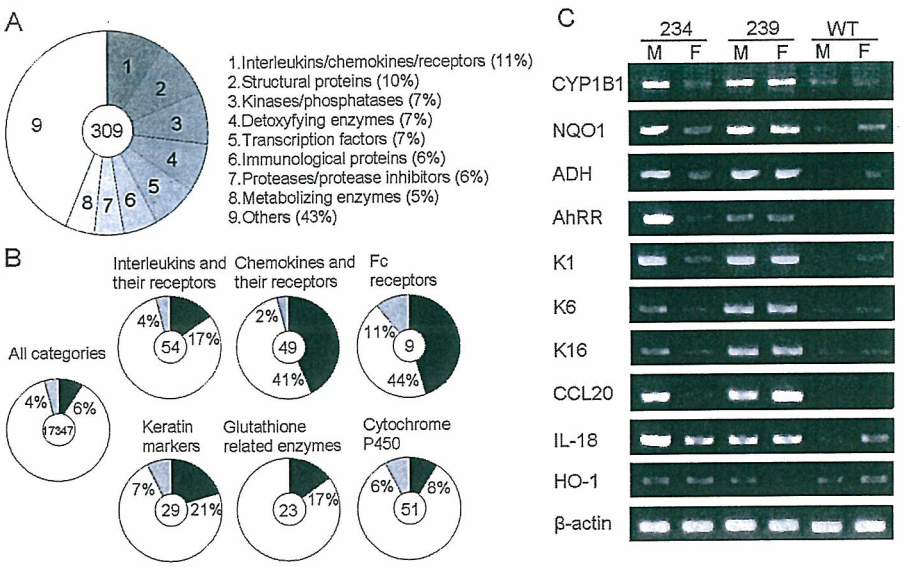

FIG. 4. Altered gene expression profiles in the skin of AhR-CA mice. (A) A total of 309 genes increased by >4-fold are categorized into nine groups. The percentage of each category is shown. (B) The ratios of the genes increased (black areas) or deceased (gray areas) by >2-fold are shown within each gene subcategory. The number written in the center of each circle graph indicates the number of genes included in that subcategory. (C) The expression levels of representative genes were examined by semiquantitative RT-PCR. cDNAs were synthesized from the skin RNAs of 10-day-old AhR-CA and wild-type mice. The corresponding line numbers are shown at the top. M, male; F, female; WT, wild type.

genic mice using adult Line 239 mice. Since local inflammation is often accompanied by systemic immune imbalance, we assessed whether the AhR-CA transgenic mice display any signs of disturbance of the immune system, particularly, imbalance between Th1 and Th2 cells. Differentiated $CD4^+$ lymphocytes (helper T cell) residing in spleen are divided into two populations, Th1 cells and Th2 cells, based on the potential ability of cytokine production. IFN-γ and IL-2 are the cytokines preferentially produced by Th1 cells, and IL-4 and IL-5 are the ones produced by Th2 cells when they are stimulated (e.g., by anti-CD3 antibody).

To examine the balance between Th1 and Th2 cell populations, we first measured the serum concentration of immunoglobulin and found that the concentrations of both IgG1 and IgE in the sera of Line 239 mice were markedly elevated compared to those in the wild-type littermates (Fig. 5A). Since the production of IgG1 and IgE is promoted by the cytokines derived from Th2 lymphocytes, it was strongly suggested that a Th2 cell-mediated immune response was predominant in symptomatic mice and that systemic immune balance was secondarily affected by epidermal proinflammatory responses.

To further address this point, we then isolated and cultured splenocytes of adult Line 239 mice and measured the cytokine production. Consistent with our current results, splenocytes from the symptomatic mice produced much more IL-4 and IL-5, which were secreted from Th2 cells, in response to immobilized anti-CD3 antibody (Fig. 5B). On the other hand, the production of IL-2 and IFN-γ (secreted by Th1 cells) was lower in the Line 239 mice than in wild-type mice. Taken together, we concluded that overexpression of AhR-CA gives rise to high serum IgE and IgG1 levels and a dominant Th2 response. It is noteworthy that these immune responses are often common background phenotypes accompanying atopic dermatitis (19) and a frequent consequence of exposure to environmental xenobiotics, including diesel exhaust particles (8).

DISCUSSION

We demonstrated here that keratinocyte-targeted overexpression of the AhR-CA protein represents an effective way of generating skin lesions that mimic atopic dermatitis and PAH contact hypersensitivity (8, 19). We successfully generated inflammatory skin lesions with no systemic or topical application of any exogenous chemicals, but by purely activating AhR-mediated transcription in epidermal keratinocytes. Our microarray result showed that many inflammation-related genes were actually upregulated, which is a good reflection of our unique strategy. These results clearly indicate that activation of the AhR signaling pathway itself is sufficient to initiate the inflammatory disorders.

The K14 promoter was reported to be active in the whole layers of epidermal keratinocytes, including follicular keratinocytes, when examined in transgenic mice (11). This expression pattern was consistent with that of CYP1A1 examined here (see Fig. 3H) and similar to that of endogenous AhR in skin (J. Mimura and Y. Fujii-Kuriyama, unpublished observations). Thus, AhR-CA expression driven by the K14 promoter was not merely ectopic but rather mimicked endogenous AhR distribution, allowing an interpretation that AhR-CA mice reflect a condition where AhR ligands are applied to the skin without operating AhR-independent action.

Transgenic mice expressing AhR-CA under the regulation of the mouse immunoglobulin heavy-chain enhancer showed an elevated risk in the spontaneous development of stomach tumors (2) and chemical hepatocarcinogenesis (23). When AhR-CA was expressed in mice under the regulation of T

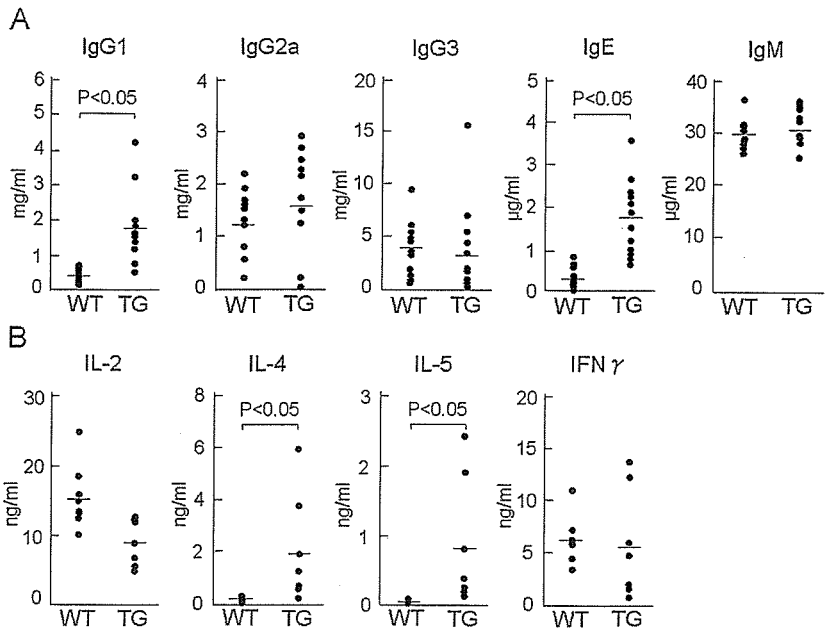

FIG. 5. Spontaneous deviation of splenic lymphocytes into Th2 cells. (A) High serum levels of IgE and IgG1 in AhR-CA mice. Sera from the AhR-CA mice (n = 13) and their wild-type littermates (n = 12) aged from 7 to 14 weeks were sampled. The serum levels of various types of immunoglobulins (IgG2a, IgG3, IgM, IgE, and IgG1) were measured by ELISA. Differences in the levels of IgE and IgG1 are statistically significant between the transgenic mice and controls ($P < 0.05$). (B) Increased production of IL-4 and IL-5 from the anti-CD3 stimulated splenic lymphocytes. Splenic lymphocytes were prepared from the AhR-CA mice (n = 7) and their wild-type littermates (n = 8) aged from 7 to 14 weeks and then stimulated with anti-CD3 antibody. The cytokine concentrations (IL-4, IL-5, IFN-γ, and IL-2) in each supernatant were determined by ELISA. Differences in the levels of IL-4 and IL-5 are statistically significant between the transgenic mice and their controls ($P < 0.05$).

cell-specific CD2 promoter, the number of thymocytes was decreased and immunization-induced T-cell or B-cell expansion was suppressed (26). Importantly, none of these reports described the development of inflammation in any tissues. In contrast, the present results provide convincing evidence that the local activation of the AhR is critical for provoking the inflammatory responses in the tissues involved. The patchy skin lesions observed in Line 234 females nicely support this contention. Thus, avoidance of local exposure to PAHs should be effective in preventing inflammatory disorders mediated by PAHs.

The development of inflammatory skin diseases has been suggested to be the result of interactions between the immune system and skin-derived molecules (18, 25). Indeed, it was previously suggested that topically applied PAHs exert a systemic influence on immune organs (16). Although the precise mechanism remains to be elucidated, we speculate that this either occurs as a direct effect of PAHs on the immune cells after percutaneous absorption or by the secondary activation of immune organs following proinflammatory responses of the skin. The AhR-CA transgenic mice displayed skin lesions accompanied by the systemic immune imbalance shifted toward the Th2 cell predominance, which could be the secondary effect of the epidermal proinflammatory responses mediated by AhR-CA protein. In addition, the direct activation of the AhR pathway in the immune organs also seemed to be responsible for the full completion of the pathological state of the transgenic mice. Consistent with the previous observation of K14 promoter activity (9), we found the additional expression of AhR-CA in the thymus (data not shown), which may have contributed to the altered immune system together with cytokines and chemokines derived from the skin lesions.

There is an interesting discrepancy between the onset of apparent inflammation in histology and that of inflammation-related gene induction. The former coincides with the postnatal period around the weaning age, and the latter starts within the late fetal stage in utero. Although the precise reason why the skin lesions develop only after birth remains unknown, we speculate that maturation of the immune systems may be required before the primary triggering signals originated from the keratinocytes come into effect for the progression of the inflammatory process. The development and maturation of skin itself might also be required.

Scratching behavior seems to exacerbate the progression of the skin lesions, ending in the massive loss of fur coats. Although prominent acanthosis and hyperkeratinization with dermal infiltration of lymphocytes and polymorphonuclear cells were commonly observed in both Line 239 and Line 234 mice, the severe scratching and dramatic skin lesions were prominently observed in Line 239 mice but not in Line 234 mice. The male mice of Line 234 die around the weaning, so they do not live until they were able to scratch their skin. Female mice of Line 234 scratch their skin only mildly; probably the itching may not be so strong because of the spotted inflammation in their skin. Hence, the further evolvement of the skin lesions seems to correlate well with the presence of scratching behavior.

One important question remained is which genes are responsible for triggering the skin inflammation in response to the constitutively active AhR. Among the various genes in-

duced in the transgenic skin, IL-18 and CCL20 are good candidates for triggering the inflammatory responses. IL-18 is known to induce inflammatory skin diseases similar to atopic dermatitis when expressed in mice through the K14 promoter (17). Similarly, if secreted from keratinocytes, CCL20 would be able to trigger skin inflammation by directing the migration of inflammatory cells (34). Indeed, there has been a strong suggestion of a correlation between CCL20 and atopic dermatitis (24). Multiple XRE found in the upstream regions of the *IL-18* and *CCL20* genes imply that these genomic regions are responsive to the activated AhR. However, other proinflammatory genes that do not contain an XRE in the proximal promoter region may also have contributed, since recent studies revealed the XRE-independent AhR function as a coactivator for other transcription factors (27). Further investigation is necessary for elucidation of the primary target genes that are activated by AhR-CA and trigger the inflammatory responses.

Intriguingly, the phenotype of the skin lesions, with remarkable cell infiltration and itching, resembles the contact dermatitis triggered by other PAHs (5) more than the chloracne triggered by dioxins (6, 37), implying the operation of distinct mechanisms underlying the two pathological conditions. A previous report showed that binding sequences are different between dioxin-liganded AhR and PAH-liganded AhR (21a), suggesting that a profile of gene activation by AhR after dioxin exposure could be different from that by AhR after PAH exposure. We surmise that a profile of gene activation by AhR-CA might be more similar to that by PAH-liganded AhR. Tissue delivery and distribution of dioxins and PAHs might be another factor to generate the phenotypic difference. It seems likely that AhR activation triggered in PAH contact dermatitis predominantly occurs in keratinocytes, as seen in AhR-CA mice we generated in the present study.

The present study demonstrates for the first time the primary contribution of AhR-mediated transcriptional activation to the development of inflammatory diseases. Since no exogenous chemical was required to trigger the inflammatory responses in this model system, activation of the downstream genes under AhR regulation is most likely to be responsible. Our findings suggest that the induction of AhR target genes is one of the central mechanisms of PAH-mediated inflammatory diseases. Based on these findings, the possibility emerges that blocking AhR signals may help to relieve the allergic symptoms, since xenobiotic exposure exacerbates allergic diseases through the AhR pathway.

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Review

Molecular mechanisms of AhR functions in the regulation of *cytochrome P450* genes

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Abstract

AhR, a ligand-activated transcription factor, mediates xenobiotic signaling to enhance the expression of target genes, including drug-metabolizing cytochrome P450s. The recent development of several new techniques, including chromatin immunoprecipitation and RNA interference, has expanded and deepened our knowledge of AhR function in the xenobiotic signal transduction. In this review, we briefly summarize our current understanding of the activation and inactivation of AhR activities and discuss the future directions of AhR research.

Keywords: Aryl hydrocarbon receptor; Cytochrome P450; Transcription factor; Coactivator; Gene regulation

Cytochrome P450 (CYP) is a superfamily of hemoproteins, composed of more than 3000 molecules and distributed across species ranging from bacteria to vertebrates. These proteins catalyze the monooxygenation of various endogenous and exogenous substrates [1,2]. Superfamily members are classified according to the similarity of their primary structures; members of families 1, 2, 3, and 4 are mainly involved in the metabolism of exogenous chemicals, including drugs, food additives, and environmental pollutants. CYPs are typically inducible; specific forms of CYPs are induced in response to the administration of certain chemicals [3–5].

Recently, the molecular mechanisms governing the inducible expression of CYPs have been successfully elucidated, including the inducers, *cis*-acting DNA elements, cognate transcription factors, and coactivators. The inducible expression of the CYP1 family is regulated by a heterodimer of the aryl hydrocarbon receptor (AhR or dioxin receptor) and the aryl hydrocarbon receptor nuclear translocator (Arnt), which contain a bHLH-PAS structural motif [5,6]. Expressions of the CYP2, 3, and 4 family members are controlled by other transcription factors (CAR, PXR, and PPAR) of the nuclear receptor (NR) superfamily, which have a characteristic zinc finger motif different from that in AhR [3–5].

The specific involvement of these receptor-type transcription factors in the induction of certain CYPs has been confirmed by gene-engineering technology, including gene-knockout methods [3,6–8]. The recent development of new techniques, including small interference RNA (siRNA) and chromatin immunoprecipitation (Chip) analyses, has greatly expanded our knowledge of the molecular mechanisms controlling the inducible expression of drug-metabolizing CYPs [9]. In this short review, we summarize the recent advances in the study of AhR activation and inducible expression of CYP1 and some other CYP families. The mechanisms of NR-related inducible expression of CYP2, 3, and 4 families are addressed in a number of excellent review articles [10–12].

Activation of AhR

Normally, AhR exists in a dormant state within the cytoplasm in association with a complex of HSP90, XAP2, and p23. Upon ligand binding, AhR in the complex is activated by a conformation change that exposes a

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nuclear localization signal(s) (NLS). The ligand-activated AhR in the complex translocates into the nucleus and forms a heterodimer with the closely related Arnt protein already present in the nucleus by dissociating from the complex [5,6].

Structure–activity relationship studies examining the binding activity of AhR ligands using a large number of halogenated aromatic hydrocarbons (HAHs) and polycyclic aromatic hydrocarbons (PAHs) suggest that the presumed AhR binding pocket accepts planar ligands with maximal van der Waal's dimensions of $14 \times 12 \times 5$ Å [13,14] (Fig. 1). Some electronic and thermodynamic properties of ligands appear to be important for a high binding affinity, although formulation of the exact structure necessary has yet to be defined [14]. 2′,3′,7′,8′-Tetrachlorodibenzo-*p*-dioxin (TCDD) and indolo[2,3-*b*]carbazole (ICZ) are the most potent inducers of CYP1 expression. AhR, which is well conserved from invertebrates like *Caenorhabditis elegans* and *Drosophila melanogaster* to vertebrates [15], mediates the majority of pharmacological and toxicological effects on host animals elicited by those xenobiotics. This high degree of evolutionary conservation among species suggests that AhR plays an important physiological role in homeostasis and/or development. In support of this hypothesis, many endogenous compounds with chemical properties different from those of the known high affinity xenobiotic ligands, such as tryptamine and indole acetic acid [16], bilirubin and biliverdin [17], and lipoxin A4 [18], have been isolated as potential natural ligands of AhR (Fig. 1). These compounds have relatively low binding affinities for AhR in comparison to those of TCDD, 2,3,7,8-tetrachlorodibenzofuran (TCDF), and ICZ. Of the tryptophan-derived natural AhR ligands like indirubin and indigo, 6-formylindolo[3,2-*b*]carbazole (FICZ), a tryptophan photoproduct, has a very high affinity for AhR, comparable to that of TCDD (Fig. 1). FICZ is formed in cell culture medium exposed to light in the presence of riboflavin [19]. Identification of all these compounds as active ligands for AhR was conducted by examining their ability to induce *CYP1A1* gene expression in cultured cells or by measuring their xenobiotic response element (XRE)-binding activity by gel mobility shift assay (GMSA). Although these chemicals have the potential to activate AhR activity, identification of a true physiological ligand for AhR would require to clarify how the activation of AhR by these naturally occurring ligands is associated with specific physiological functions.

Recently, a number of papers have reported that AhR is activated in the absence of obvious ligands in Hepa 1clc7 cells [20], human keratinocytes [21], 10T1/2 fibroblasts [22], and HaCaT cells [23] grown under specific culture conditions. When Hepa-1 cells and human keratinocytes were grown in suspension, CYP1A1 mRNA was induced without the addition of AhR ligands in an AhR-dependent manner at levels similar to that seen following TCDD treatment [20,21]. Suspension cultures of several C3H10T1/2 fibroblast clonal sub-lines that contain an integrated AhR-responsive reporter gene exhibited a time-course and levels of reporter activation and endogenous CYP1B1 induction that paralleled TCDD stimulation in confluent monolayer culture. Loss of cell–cell contacts at low culture densities also activated the expression of the

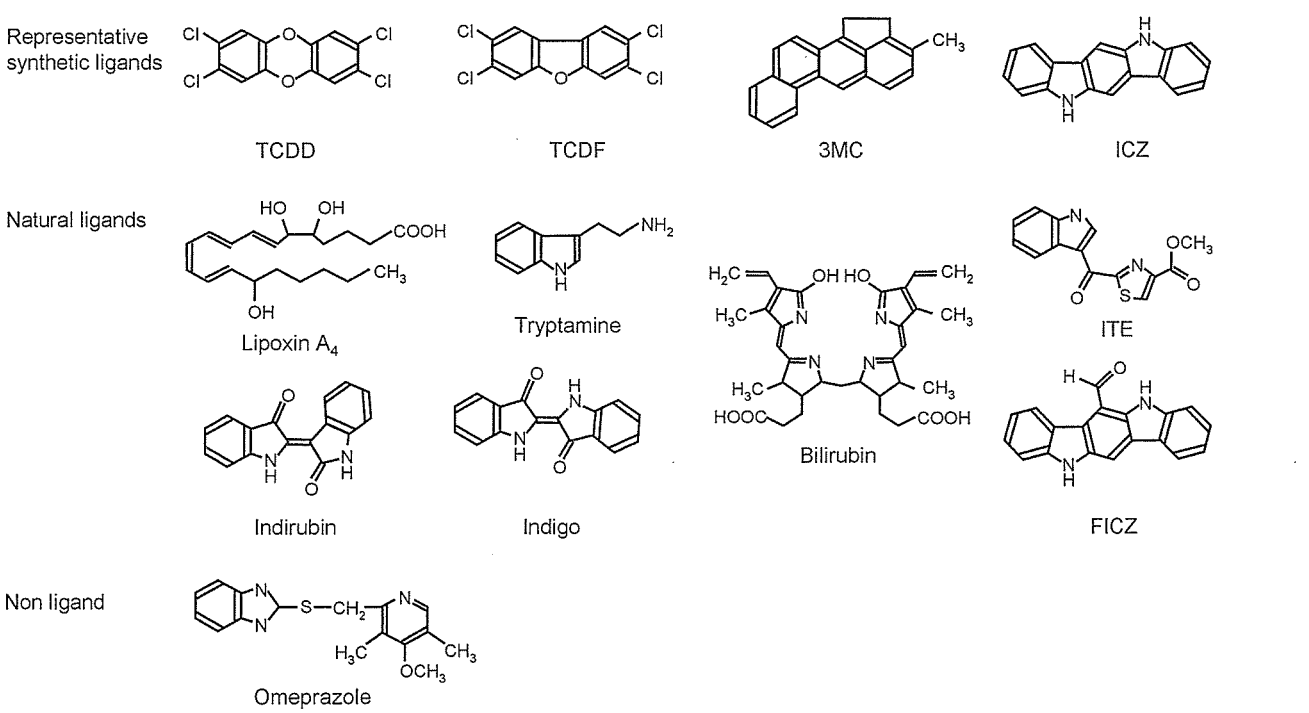

Fig. 1. Chemical activators of AhR. TCDD: 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, TCDF: 2,3,7,8-tetrachlorodibenzofuran, 3MC: 3-methylcholanthrene, ICZ: indro[3,2-*b*]carbazole, ITE: 2-(1′*H*-indole-3′-carbonyl)-thiazole-4-carboxylic acid methyl ester, FICZ: 6-formylindolo[3,2-*b*]carbazole.

reporter at levels comparable to TCDD stimulation of confluent cells in a manner independent of cell cycle changes. Suspension culture and TCDD treatment induced comparable AhR nuclear translocation and AhR/Arnt complex formation [22]. Culture of a keratinocyte cell line, HaCaT, at low cell densities or at confluence in Ca^{2+}-deficient S-MEM induces the nuclear accumulation of AhR in association with enhanced expression of a reporter gene whose expression is driven by XRE sequences [23]. These findings suggest that disruption of cell–cell contacts or cell–cell interactions stimulates the nuclear localization and transcriptional activity of AhR via a signal transduction pathway. The nuclear accumulation of AhR is regulated by the phosphorylation of Ser68 within the nuclear export sequence (NES) of AhR. Use of specific kinase inhibitors has suggested that this phosphorylation event is catalyzed by p38 MAPK [23]. An increasing number of reports have described that phosphorylation regulates AhR activity in the physiological signaling pathway regulating cell cycle progression as well as the xenobiotic signal transduction pathway [24]. Omeprazole induces CYP1A1 in an AhR-dependent manner without binding directly to AhR [25,26]. Tyrosine kinase inhibitors, tyrphostins AG17 and AG879, selectively inhibited omeprazole-mediated AhR signaling, but did not affect TCDD-mediated induction of CYP1A1. Mutational analysis provided evidence that a Tyr320Phe mutation abolished omeprazole-dependent AhR activation, while the TCDD-dependent activation of *CYP1A1* transcription was only minimally affected. These results suggest that Tyr320 is a putative phosphorylation site on AhR activated by omeprazole in a ligand-independent manner via a signal transduction pathway that involves protein tyrosine kinases. This pathway is independent from that induced by high-affinity ligands, such as TCDD [26]. Although the protein kinases involved remain unclear, AhR can be activated in a ligand-independent manner.

Cis-acting DNA elements

The regulatory DNA elements responsible for the induction of CYP1 by polyaromatic hydrocarbons like TCDD, called XREs, were first identified by transient DNA transfection experiments using a reporter gene, whose expression was driven by the *CYP1A1* promoter [27]. Later, additional experiments introducing a variety of mutations at this locus defined the consensus sequence and designated this sequence the DRE or AhRE [5,28]. All *CYP* genes whose expression are induced by PAH or HAH, including CYP1A1, 1A2 [6,27,28], 1B1 [29], 2A8 [30], and 19 [31] (Fig. 2), carry XRE sequences within their promoters. The human *CYP1A1* and *1A2* genes, found on chromosome 15q23 [32], are arranged in head to head orientation at a distance of approximate 23 kb apart. A similar chromosomal arrangement is reported for the mouse *Cyp1A1* and *1A2* genes on chromosome 9B, the syntenic chromosome to human Ch15q23. Although both genes are similarly inducible by PAH or HAH and share a common 5′ flanking region, the regulatory mechanisms governing the expression of each are quite different. Under normal conditions, CYP1A2 is expressed at basal levels in the liver, while no basal expression of CYP1A1 is observed in this

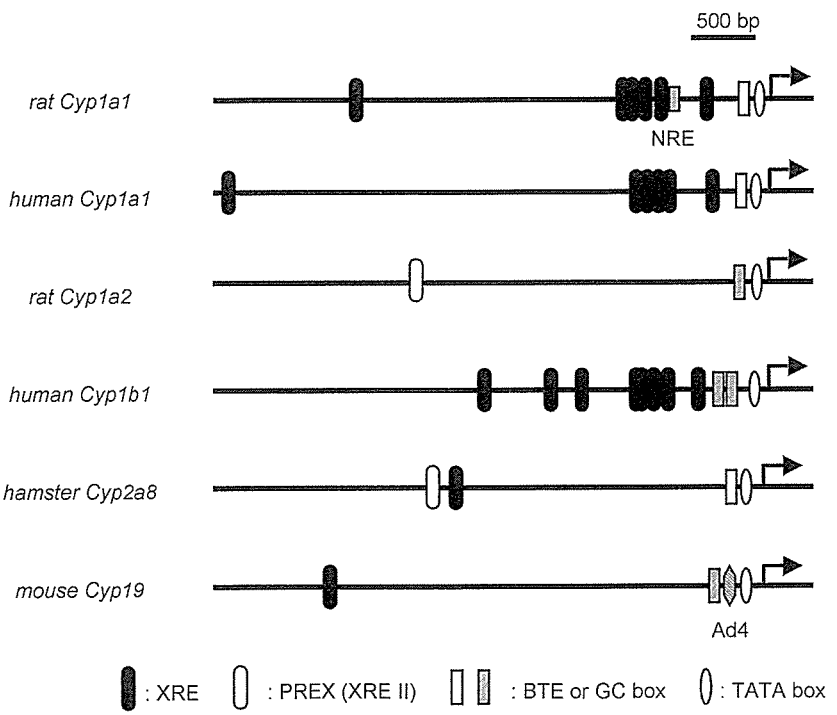

Fig. 2. Schematic representation of regulatory elements in the promoter of *CYP*s.

organ. Expression of CYP1A1 could be upregulated in multiple tissues in response to inducers, while that of CYP1A2 is restricted to the liver. In AhR-deficient mice, CYP1A1 expression is completely abolished; in these animals, although basal expression of CYP1A2 was retained in the liver, inducible expression was lost [33]. These findings clearly indicate that the expression of CYP1A1 and 1A2 is differently regulated, despite a common 5′ upstream sequence. Recently, a DNA element responsive to 3MC (XRE2: CATGN$_6$ CTTG), which is similar to a consensus DNA-binding sequence [CNRG-N$_{5\sim6}$-CNR(G/C)] recognized by the LBP-1 family, was discovered in the proximal promoter of the *CYP1A2* gene [34]. A putative factor (X) binding to this sequence was suggested by GMSA. Ligand-activated AhR/Arnt bound to the X factor as a coactivator is likely conferring inducibility of the *CYP1A2* gene in response to the inducer [34]. Coactivator-like functions of AhR/Arnt have already been reported [35,36]. An analogous sequence, designated PREX, was also identified within the promoter of the *CYP2A8* gene. This sequence acted as an inducible enhancer that cooperated with the XRE sequence in the *CYP2A8* promoter. The factor binding to PREX was identified as NF2d9 (LBP-1a) [37]. Less is known of the regulatory mechanisms governing the inducible expression of CYP1A2. In addition to the XRE sequence, a BTE (basic transcription element) sequence, which is a GC box sequence localized in the proximal promoter of *CYP1A1*, is also important to achieve a high level of CYP1A1 [38] and 2A8 [30] inducible expression. The XRE and GC box sequences frequently coexist in the promoters of xenobiotic-inducible genes, suggesting that these elements cooperatively enhance the inducible expression of these genes (Figs. 2 and 3). Another putative NF-1 binding site has also been reported within the proximal promoter of the *CYP1A1* gene [9], but no experimental evidence has addressed its functional significance. Two regulatory DNA elements are found in the promoter of *CYP19* gene, XRE and Ad4/SF-1, whose cognate binding factors, AhR and Ad4BP/SF-1, interact on the chromosomal DNA to enhance gene expression synergistically [31].

Trans-acting factor for XRE

Mouse genetics initially implicated the existence of a mediator of the xenobiotic signaling, leading to the induction of CYP1A1 expression. This mediator was later identified as a factor binding to xenobiotics, which was designated the aryl hydrocarbon receptor or AhR [39]. This factor was also dubbed the dioxin receptor (DR), due to the high avidity with which it bound TCDD. Approximately a decade later, GMSA revealed that a factor that bound the XRE sequence in a TCDD-dependent manner contained a factor which also bound TCDD directly. This XRE-binding factor behaved like AhR; both the cytoplasmic localization under normal conditions and the induction of nuclear translocation by TCDD treatment mimicked the patterns seen for AhR [40]. cDNA cloning of this XRE-binding factor revealed its molecular structure to be a DNA-binding tran-

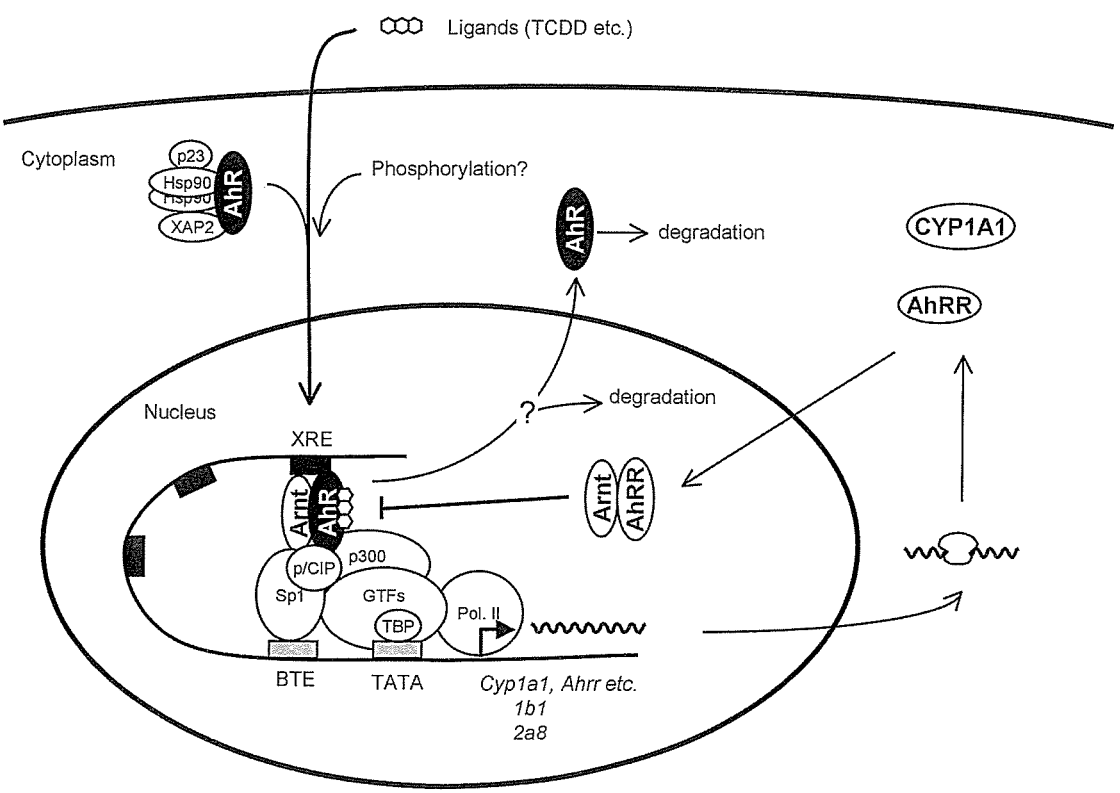

Fig. 3. A model of AhR signaling pathway.

scription factor with a bHLH motif similar to that seen for cMyc and MyoD [41,42]. Expression experiments demonstrated that AhR exogenously expressed from cDNA could be activated by TCDD or 3MC to form a heterodimer with Arnt, resulting in activation of XRE binding and transactivating activities. DNA footprint analysis and GMSA revealed that binding of Sp1 to the BTE facilitated the binding of the AhR/Arnt heterodimer to the XRE by physical interaction and vice versa, cooperatively enhancing expression of the *CYP1A1* gene [38]. The AhR/Arnt heterodimer bound to the XRE sequence and, in turn recruited CBP or P300, a HAT coactivator, to the C-terminal activation domain of Arnt [43]. Transient DNA transfection assays using a XRE-driven reporter gene demonstrated that the coactivators SRC-1 and RIP-140, the retinoblastoma protein Rb, PML, and Nedd8 interact with AhR to enhance reporter gene expression [9]. ChIP assay confirmed and extended the involvement of these coactivators in the inducible expression of *Cyp1A1*. These studies determined that the related HAT coactivators, SRC-1, NCoA-2, and p/CIP, all associate with the *Cyp1A1* enhancer region in Hepa-1 cells in a TCDD-dependent manner [44,45]. Injection of anti-SRC-1 or anti-p/CIP Abs into Hepa-1 cells abolished the TCDD-mediated induction of the reporter gene, clearly demonstrating that these factors function in AhR-mediated xenobiotic signal transduction as coactivators [45]. Overexpression of these cofactors revealed that SRC-1 and NCoA-2, but not p/CIP, interact with Arnt, while AhR interact with all three coactivators. In contrast to the interaction with CBP, the helix 2 domain of Arnt interacts with SRC-1 [9,45].

Typically, genomic DNA is closely packed into the unique units of chromosomal structure, called nucleosomes. These structures consist of 146 bp of DNA wound around a core of histone proteins, containing two molecules each of H2A, H2B, H3, and H4. Neighboring nucleosomes are associated through a short stretch of DNA covered by a H1 histone linker. Assembly of this fundamental chromosomal structure prevents the transcriptional machinery from gaining access to the genes involved. Experiments using micrococcal nuclease revealed that, in the absence of inducers, the 5′ upstream regulatory and coding regions of *CYP1A1* exist in a silent nucleosomal configuration. A nucleosome at the proximal promoter exists in a fixed position approximately −60 to −120 bp from the *CYP1A1* transcription start site, while the other nucleosomes are positioned randomly throughout the gene. TCDD treatment disrupts nucleosomes in the promoter and the transcribed portions of the gene, relieving nucleosomal repression [46].

In association with the chromosomal remodeling that occurs during drug induction, transient DNA transfection and ChIP analysis indicated the involvement of BRG1, a component of a subset of SWI/SNF ATP-dependent chromatin-remodeling complexes, in the enhancement of *Cyp1A1* gene expression by TCDD. Exogenous expression of BRG1 potentiated AhR/Arnt-mediated reporter gene expression in a TCDD-dependent fashion in Hepa-1 cells. Upon co-transfection with SRC-1, BRG1 restored the inducible expression of the endogenous *Cyp1A1* gene in the BRG-1- and hBrm-deficient SW13 and C33 cell lines in response to the inducer. An ATPase-deficient mutant of BRG-1, however, was unable to do so. ChIP analysis demonstrated that BRG-1 associates with the enhancer region of the *Cyp1A1* gene in vivo in a TCDD- and Arnt-dependent manner, suggesting the specific recruitment of BRG-1 by the AhR/Arnt heterodimer. These results indicate the importance of ATP-dependent chromatin remodeling in the inducible gene expression mediated by the AhR/Arnt heterodimer [47].

The recruitment of the Mediator/TRAP/DRIP/ARC multisubunit complex to the *Cyp1A1* promoter is also important for the transduction of xenobiotic signals that activate target gene expression, as revealed by ChIP analysis, DNA transfection, and RNAi experiments. ChIP kinetic analysis of recruitment of Med220 and CDK8 (subunits of Mediator/TRAP/DRIP/ARC) demonstrated that these factors associate with the enhancer XRE sequence rapidly and persistently after TCDD treatment. These kinetics followed shortly after those seen for binding of AhR and p/CIP coactivators to the XRE sequence. In contrast, *Pol*II bound to the promoter, but not to the enhancer. Depletion of Med220 from HepG2 cells by RNAi substantially reduced Med220 protein levels and inhibited endogenous AhR-mediated transcription from the *Cyp1A1* gene. Treatment of Hepa-1 cells with TCDD induced the binding of AhR and p/CIP to the enhancer sequence within 10 min. The binding of p300 and Med220 to the enhancer and the binding of *Pol*II to the promoter were detected after 15 min. After binding reached maximal value at 15–30 min, the degree of binding of each protein remained constant until 2 h after treatment [6,48]. Although a relatively large number of coactivators and mediators have been identified to be involved in AhR-dependent induction of CYP1A1 (Fig. 3), it seems likely that additional coactivators are also involved. As CYP1A1 is strongly induced by PAHs and HAHs, such as TCDD, and a considerable amount of information has been accumulated in regard to the transcription and chromatin remodeling factors involved, *CYP1A1* induction continues to be a good model in which to elucidate the precise mechanisms of gene regulation, including the temporal and spatial recruitment of mediators, the interactions between transcription factors, and the modification of nucleosomal structures (Fig. 3).

Degradation of the AhR

To understand the mechanisms of gene regulation, it is important to investigate both the upregulation and termination phases of inducible transcription. Recent reports have suggested that AhR is rapidly downregulated following ligand binding by degradation [49]. Experiments using the proteasome inhibitor MG132 suggested that the downregulation of AhR is mediated by the proteasome. The concentrations of AhR proteins in the nuclear frac-

tions of cultured cells are highest after 1–2 h of TCDD treatment, and then, rapidly decline with increasing time. In the presence of MG132 or lactacystin, a 26S proteasome inhibitor, TCDD treatment elicited even higher levels of AhR and Arnt proteins within nuclei at as late as 8 h. The high levels of nuclear AhR and Arnt detected in cells treated with proteasome inhibitors included increased amount of the AhR and Arnt heterodimer that is capable of binding the XRE sequence. Cells treated with a combination of TCDD and MG132 induced reporter gene expression more rapidly at a greater magnitude than cells treated with TCDD alone. The luciferase activity increased linearly throughout the 8 h time course in cells treated with TCDD and MG132, peaking at approximately a 1300-fold induction. In cells treated with TCDD alone, inducible reporter expression peaked at 5 h, with only a 100-fold induction that gradually decreased thereafter [49,50].

A number of reports investigated the subcellular location of AhR degradation. LMB is a specific inhibitor of CRM-1-mediated nuclear export. Following LMB treatment, AhR remained predominantly in the nuclei. Treatment with this inhibitor abrogated the degradation of ligand-activated AhR in the culture cells, HepG2 or Hepa-1. GMSA demonstrated that AhR in the nuclei of LMB- and TCDD-treated cells were capable of binding to the XRE sequence. These results suggest that AhR must be exported from the nucleus to be degraded [50]. A recent study using a constitutively nuclear AhR (termed DR-NLS), which contains two NLS, revealed that AhR (DR-NLS) was degraded within nuclear compartments in a 26S proteasome-dependent manner. Although the degradation of the modified AhR (DR-NLS) may differ from that of native AhR, these results suggest that AhR can be degraded within the nucleus [51,52]. Future experiment will need to address whether the AhR and Arnt heterodimer bound to the XRE sequence is degraded by proteasome while bound to the DNA or after release from the XRE sequence. The AhR repressor AhRR, whose expression is enhanced by AhR, forms a heterodimer with Arnt to replace the AhR/Arnt complex in association with the XRE sequence [53]. AhRR may play a role in releasing the AhR/Arnt heterodimer from the XRE sequence, facilitating its degradation. As the degradation of AhR is important for the regulation of AhR activity, determination of the physiological location of AhR degradation remains important for future research.

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Constitutively Active Aryl Hydrocarbon Receptor Expressed Specifically in T-Lineage Cells Causes Thymus Involution and Suppresses the Immunization-Induced Increase in Splenocytes[1]

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The aryl hydrocarbon receptor (AhR) is a transcription factor belonging to the basic helix-loop-helix-PER-ARNT-SIM superfamily. Xenobiotics, such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, bind the receptor and trigger diverse biological reactions. Thymocyte development and T cell-dependent immune reactions are sensitive targets of AhR-dependent 2,3,7,8-tetrachlorodibenzo-*p*-dioxin toxicity. However, the exact role of the AhR in T cells in animals exposed to exogenous ligands has not been clarified because indirect effects of activated AhR in other cell types cannot be excluded. In this study, we generated transgenic (Tg) mice expressing a constitutively active mutant of AhR under the regulation of a T cell-specific CD2 promoter to examine AhR function in T cells. The mRNAs of the constitutively active mutant of AhR and an AhR-induced gene, CYP1A1, were expressed in the thymus and spleen of the Tg mice. The transgene expression was clearly detected in the thymocytes, CD4, and CD8 T cells, but not in the B cells or thymus stromal cells. These Tg mice had a decreased number of thymocytes and an increased percentage of CD8 single-positive thymocytes, but their splenocytes were much less affected. By contrast, the increase in number of T cells and B cells taking place in the spleen after immunization was significantly suppressed in the Tg mice. These results clearly show that AhR activation in the T-lineage cells is directly involved in thymocyte loss and skewed differentiation. They also indicate that AhR activation in T cells and not in B cells suppresses the immunization-induced increase in both T cells and B cells. *The Journal of Immunology,* **2005, 174: 2770–2777.**

Xenobiotics, such as polycyclic aromatic hydrocarbons and halogenated aromatic hydrocarbons, bind and activate the aryl hydrocarbon receptor (AhR),[3] a transcription factor belonging to the basic helix-loop-helix-PER-ARNT-SIM (bHLH-PAS) superfamily (1, 2), and elicit diverse biological and physiological responses (3–6). These findings suggest that the AhR functions physiologically as a ligand-dependent transcription factor, whereas the endogenous ligands and intrinsic role of the AhR have yet to be identified. The decreased fertility and abnormalities found in various organs, including the liver, spleen, vascular structures, ovary, mammary gland, and bone marrow lymphocytes, in AhR-deficient mice (7–12) also imply intrinsic roles of the AhR in normal developmental processes. In the absence of ligands, the AhR exists in the cytoplasm in an inactivated form complexed with a dimer of heat shock protein 90 and the immunophilin homologue hepatitis B virus X-associated protein 2 (13). Upon binding with ligands, such as its most potent ligand, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), the AhR becomes activated, dissociates from the protein complex, and translocates into the nucleus, where the receptor dimerizes with another basic helix-loop-helix-PAS transcription factor, aryl hydrocarbon receptor nuclear translocator (ARNT). The AhR/ARNT heterodimer specifically binds DNA sequences, called xenobiotic responsive elements (XREs), distributed in the enhancer regions of various genes, including one of the most sensitive targets, CYP1A1, and modulates their expression (14). The receptor complex also interacts with various nuclear proteins, such as retinoblastoma, NF-κB, and estrogen receptors (15–17). However, determination of the functions of the AhR requires identification of the genes and proteins that it modulates and the cell types in which the individual biological or physiological reactions occur.

The immune system is one of the sensitive targets of TCDD (6). Although a major portion of TCDD toxicities, such as thymus involution, suppressed CTL activity, and reduced Ab production, have been demonstrated to be mediated through the AhR by studies in AhR-deficient mice (18–20), the precise mechanisms of AhR function, including the primary cellular targets and biological reactions involved in these toxic effects, remain to be clarified. The thymus involution induced by administration of TCDD or other AhR ligands to mice is characterized by decreases in tissue weight and cell number that are mainly attributable to a decrease in $CD4^+CD8^+$ double-positive (DP) cells, the predominant population of thymocytes. Skewing of thymocyte differentiation toward CD8 single-positive (SP) T cells is another peculiar feature of the

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[3] Abbreviations used in this paper: AhR, aryl hydrocarbon receptor; PAS, PER-ARNT-SIM; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; ARNT, aryl hydrocarbon receptor nuclear translocator; XRE, xenobiotic responsive element; DP, double-positive; SP, single-positive; FTOC, fetal thymus organ culture; Tg, transgenic; CA-AhR, constitutively active mutant of AhR; h, human; DIG, digoxigenin; 7-AAMD, 7-aminoactinomycin D; DN, double negative.

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response to TCDD exposure (18, 21, 22). All of these features are reproduced in vitro by direct addition of TCDD to fetal thymus organ culture (FTOC) (19, 23, 24), indicating that the target cells responsible for the alterations are present in the thymus. The results of previous studies that have included histological examination have led to the hypothesis that thymic stromal cells, and not thymocytes themselves, are the direct targets of TCDD that induce thymus involution (25). This hypothesis was supported by a study showing that the stroma of a fetal thymus reaggregation culture treated with an AhR-binding halogenated aromatic hydrocarbon, not the thymocytes, induced thymus involution (24). By contrast, a recent study in which chimeric mice having AhR-deficient hemopoietic cells and wild-type stromal cells or vice versa were exposed to TCDD demonstrated that the AhR in the hemopoietic compartment, that is in the thymocytes or their precursor cells, is responsible for the TCDD-induced thymus involution (18).

The results of other studies have also shown or suggested that the AhR in T cells plays an essential role in TCDD-induced immunotoxicity. A recent study by Kerkvliet et al. (26) in a mouse graft-vs-host model injected with $AhR^{+/+}$ or $AhR^{-/-}$ T cells showed that AhR activation in T cells is critical to the suppression of CTL activity by TCDD. In our own study examining the effect of TCDD on OVA-specific Ab production in mice (27), TCDD exposure suppressed the increase in T cell number in the spleen and production of IL-2 and Th2-type cytokines before the inhibition of Ag-induced Ab production, suggesting that the AhR activation in T cells causes suppression of T cell activation and subsequent immune reactions leading to Ab production. However, it is difficult to determine the specific role of AhR activation in T cells alone in TCDD-exposed mice, because the AhR in all cell types, including B cells and APCs, is simultaneously activated, and indirect effects cannot be excluded. Chimeric mouse models and a T cell transfer system produced by using AhR-deficient mice or their cells are very useful tools for studying the primary cell target of TCDD, but they are inconvenient, because generation of chimeric mice and reconstitution by T cell transfer require highly specialized techniques. In addition, when chimeric mice are used, it must be borne in mind that their hemopoietic cells contain precursor cells not only for T cells but for B cells and APCs as well (28).

To investigate the role of AhR activation in TCDD-induced immunotoxicity, in the present study we generated transgenic (Tg) mice that specifically express a constitutively active mutant of AhR (CA-AhR) in T-lineage cells by expressing a CA-AhR with a minimal deletion in the PAS-B domain (29) under the regulation of a CD2 promoter. The AhR mutant constitutively localizes to the nucleus, heterodimerizes with ARNT, and activates transcription by binding XRE sequences in a ligand-independent manner (29, 30). The results of the present study demonstrate that AhR activation in T-lineage cells alone directly induces the thymocyte changes. They also show that the increase in number of splenocytes after immunization is suppressed in the Tg mice, whereas resting splenocytes in nonimmunized mice are much less affected, suggesting that the AhR plays a role in the growth of activated and proliferating T cells.

Materials and Methods

Generation of Tg mice

The CA-AhR expression construct (VA hCD2-CA-AhR) was generated by subcloning PAS B-domain-deleted mouse AhR cDNA with poly(A) signal (29) into the *EcoRI/BamHI* site of the VA human CD2 (hCD2) minigene, an improved version of a human CD2 minigene-based vector (31). Tg founder mice were obtained by microinjecting the transgene expression construct into C57BL/6J × DBA/2 eggs as described previously (32). In some lines, VA hCD2-GFP was coinjected with VA hCD2-CA-AhR. One line carrying both CA-AhR and GFP constructs (line A) and two lines with the CA-AhR construct alone (lines K and N) were chosen for further studies and subsequently were backcrossed into C57BL/6J mice. Founders and subsequent littermates were genotyped by PCR of tail DNA using primers for VA hCD2-CA-AhR (5′-GAACAGAGAGTTTGTCCAGC-3′, located in hCD2 promoter, and 5′-CTTCCAAAGGTAAGCATAAGAGTC-3′, located in N terminus of CA-AhR). Integrated CA-AhR copy number was determined by Southern blot analysis. Genomic DNA from a tail sample was digested with *EcoRI* and *PstI*, separated by agarose gel electrophoresis, blotted onto a Hybond filter (Amersham), and hybridized with a digoxigenin (DIG)-labeled probe. The DIG-labeled probe was synthesized from the *HincII* digestion fragment of pEB6CAG-CA-AhR-GFP (29) with a DIG-high prime DNA labeling and detection starter kit I (Roche Diagnostics) and was detected with CSPD as a substrate according to the manufacturer's instructions. Heterozygous ($CA\text{-}AhR^{+/-}$) mice were used for experiments after crossing into C57BL/6 mice for two to six generations. Their nontransgenic ($CA\text{-}AhR^{-/-}$) littermates (designated as wild type) were used as controls.

Cell preparation

Single cell suspensions of thymus and spleen were prepared by forcing cells in RPMI 1640 medium supplemented with 12 mM HEPES (pH 7.1), 0.05 mM 2-ME, 100 U/ml penicillin, 100 μg/ml streptomycin, and 10% FCS (complete medium) through a stainless-steel mesh. Spleen cells and bone marrow cells prepared from thigh bones were treated with ammonium chroride/EDTA solution (0.83% NH_4Cl, 0.1% $KHCO_3$, 0.37% EDTA (pH 7.4)) for 2 min at room temperature to eliminate RBCs and then were washed with PBS (33). Cells were counted with a hemocytometer after staining with trypan blue.

RT-PCR

Total RNA was isolated from cells or tissues with an RNeasy Mini kit (Qiagen). After checking the quality of the RNA by electrophoresis, RT-PCR was performed with an RNA LA PCR kit (AMV) ver1.1 (TaKaRa Biomedicals) according to the manufacturer's instructions. The amplification was conducted by heating at 94°C for 2 min, cycling at 94°C for 30 s, 60 or 66°C for 30 s, and 72°C for 30 s, and then extension at 72°C for 10 min after the final cycle. The primer sequences and annealing temperatures for each gene are shown in Table I. The primers for detecting mRNA expression of CA-AhR were designed to span the region coding for PAS B domain to distinguish PCR products between CA-AhR and wild-type AhR. The PCR products were separated with a 1.2% Synergel (Diversified Biotech) containing 0.5 μg/ml ethidium bromide, and the gel images were captured and visualized using an Electrophoresis Documentation and Analysis System 290 (Eastman Kodak).

Flow cytometry

Cells were stained with mAbs against lymphocyte surface markers or streptavidin-allophycocyanin (BD Pharmingen) for 20 min on ice. After staining, the cells were washed, treated with 7-aminoactinomycin D (7-AAMD; Sigma-Aldrich) to label dead cells, and measured with a FACSCalibur (BD Biosciences). Live cells were gated and analyzed (22). The following mAbs, all purchased from BD Pharmingen, were used: PE-conjugated anti-CD4 (anti-CD4-PE, clone GK-1.5), FITC-conjugated anti-CD8

Table I. *List of primers used for RT-PCR*

Description	Primer Sequence (5′-3′)	Annealing Temperature (°C)	Product Size (bp)
CA-AhR	TTACCTGGGCTTTCAGCAGT	66	506
	AACTGGGGTGGAAAGAATCC		
CYP1A1	CCATGACCGGGAACTGTGG	60	344
	TCTGGTGAGCATCCTGGACA		
Adseverin	GTGCTTCTAAGCATTTCCCC	60	121
	GAGTGAATGGCATCCAAGTG		
CD4	AAGGGCTCTCCCTGAGAGTC	60	104
	AAAGAGGAAAAAAGGGGAAGG		
Spatial	GAAGGTGACAGCGAAAATCA	60	112
	AAGGCATTAGACAGGTTGGG		
β-Actin	GAGGCCCAGAGCAAGAGAG	60	225
	GGCTGGGGTGTTGAAGGT		
HPRT	GCTGGTGAAAAGGACCTCT	60	249
	CACAGGACTAGAACACCTGC		

(anti-CD8-FITC, clone 53-6.7), biotinylated anti-CD8 (anti-CD8-biotin, clone 53-6.7), anti-CD3-PE (clone 145-2C11), anti-CD19-biotin (clone ID3), anti-B220-FITC (clone RA3-6B2), anti-CD127(IL-7Rα)-biotin (clone B12-1), and anti-CD61-PE (clone 2C9.G3). Biotinylated rat IgG2a was used as an isotype-matched control.

Immunization

OVA (albumin, chicken egg, grade VII) was purchased from Sigma-Aldrich. Alum-precipitated OVA (OVA/alum) was prepared as follows (27, 34). OVA (1 mg/ml) in PBS was mixed with an equal volume of 9% (w/v) $AlK(SO_4)_2$, and pH of the mixture was adjusted to 6.5 with KOH. The precipitate was washed three times with PBS and then resuspended in PBS at 0.5 mg/ml. Mice were i.p. immunized with the OVA/alum (100 μg OVA/mouse).

TCDD treatment

TCDD (50 μg/ml in nonane) purchased from Cambridge Isotope Laboratories was diluted with corn oil to adjust it to a dose volume of 10 μl/g body weight. TCDD was administered to the mice orally.

Fetal thymus organ culture

Line A heterozygous Tg mice backcrossed into C57BL/6J mice for five generations were mated, and homozygous CA-AhR$^{+/+}$ Tg mice were obtained. Male CA-AhR$^{+/+}$ mice were mated with female C57BL/6J mice, and thymuses were collected from fetuses on gestation day 16.5. One or two lobes of the thymuses were placed on a nitrocellulose filter (45-μm pore size) set in a 24-well culture plate with 1 ml of complete medium and were cultured for 4 days (35). To deplete them of thymocytes, the lobes were cultured in the presence of 1.35 mM 2-deoxyguanosine (Sigma-Aldrich) for 4 days (36, 37).

Results

Generation of T cell-specific CA-AhR Tg mice

We used the VA hCD2 vector to generate Tg mice expressing a CA-AhR mutant (Fig. 1*A*) specifically in T-lineage cells. We chose one line carrying both CA-AhR and GFP constructs (line A) and two lines with only the CA-AhR construct (lines K and N) functioning under the control of the VA hCD2 vector for the subsequent experiments. The transgene-positive mice were mated with C57BL/6 mice and maintained as heterozygotes. Heterozygous mice were used in all experiments unless otherwise specified, and their nontransgenic (CA-AhR$^{-/-}$) littermates (designated as wild type) were used as controls. Integrated CA-AhR copy numbers were determined by Southern blotting to be 2 for line A, 6–7 for line K, and 9–11 for line N. All of the lines were fertile, exhibited a normal sex ratio at birth, showed no increase in mortality after birth, and appeared healthy.

Fig. 1*B* shows CA-AhR mRNA expression in various organs in line A Tg mice. CA-AhR mRNA was detected in the thymus and spleen as expected, and was also found in the lung and, to a very minor extent, in the kidney. Expression of the AhR-responsive gene CYP1A1 was also detected in the thymus and spleen in the Tg mice in contrast with their wild-type littermate mice (Fig. 1*C*). In the lung, CYP1A1 mRNA was detected in the wild-type mice. The lung is reported to express the highest level of AhR mRNA among the tissues examined, including the thymus and spleen, in the mice (38). Recently, endogenous ligand was isolated from porcine lung (39). Thus, the lung may contain abundant AhR and endogenous ligand may activate the receptor and induce CYP1A1. The expression of CYP1A1 in the lung was further increased in the Tg mice (Fig. 1*C*). Expression of CA-AhR and CYP1A1 mRNA was also confirmed in the thymus and spleen of lines K and N (see Fig. 4).

Expression of the transgene in immune cells was measured by flow cytometry analysis of the GFP expression in line A Tg mice (Fig. 2). Thymocytes showed a broader peak of the GFP-positive population (Fig. 2*A*). CD4 and CD8 T cells in the spleen were confirmed to be GFP-positive, and B cells did not express GFP (Fig. 2*B*).

We then investigated whether the bone marrow cells of the CA-AhR Tg mice expressed the transgene and, as shown in Fig. 3, the CD3$^-$CD127$^+$ (IL-7Rα-expressing) lymphocyte progenitor fraction (40) was found to be faintly GFP-positive (Fig. 3*C*, R3). Although a previous study reported that the VA hCD2 vector functions in megakaryocytes as well as T-lineage cells (32), CD61$^+$ megakaryocytes (41) in the bone marrow did not express the transgene (Fig. 3*C*, R4 and R5).

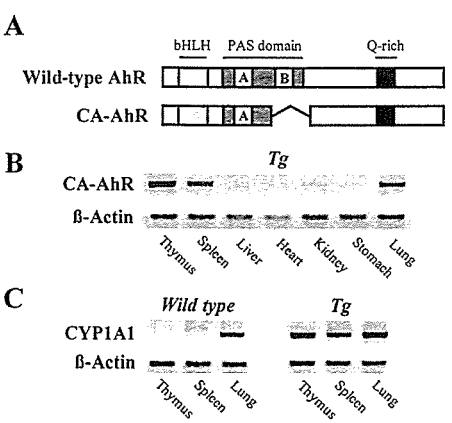

FIGURE 1. Generation of T cell-specific CA-AhR Tg mice. *A*, Schematic representation of the wild-type mouse AhR and the CA-AhR mutant lacking the minimal PAS B motif. *B*, Different tissues from line A heterozygous Tg mice were examined for CA-AhR mRNA expression by RT-PCR. C, Functional activation of the CA-AhR was confirmed by detection of CYP1A1 expression by RT-PCR.

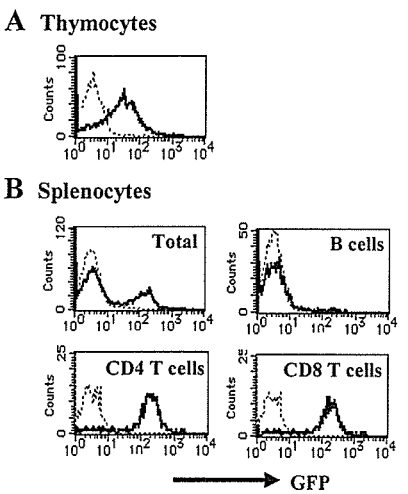

FIGURE 2. Transgene expression in thymocytes and splenocytes. *A*, Thymocytes prepared from line A heterozygous mice were stained with 7-AAMD and analyzed with a FACSCalibur flow cytometer. 7-AAMD-negative live cells were gated and expression of coinjected GFP was analyzed. *B*, Splenocytes from line A heterozygous mice were stained with a combination of anti-CD19-biotin/streptavidin-allophycocyanin and anti-CD3-PE or a combination of anti-CD8-biotin/streptavidin-allophycocyanin and anti-CD4-PE and then were stained with 7-AAMD and analyzed with a FACSCalibur. CD4 T cells, CD8 T cells, and CD19$^+$ B cells in the 7AAMD-negative live cells were gated, and GFP expression was analyzed. The staining obtained in Tg mice is represented by the bold line, and the staining in wild-type mice is represented by the dotted line.

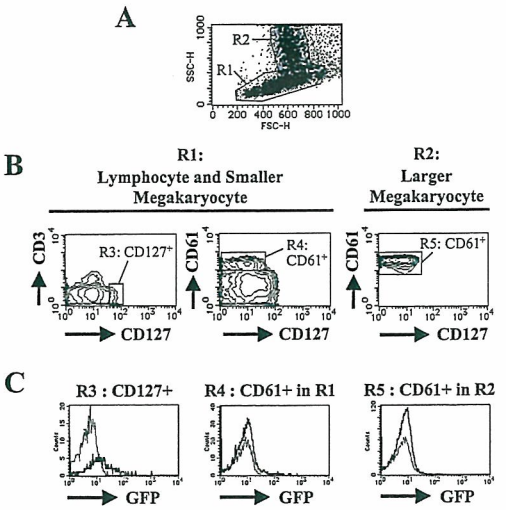

FIGURE 3. The transgene is faintly expressed in CD127$^+$ cells in the bone marrow. Bone marrow cells from line A heterozygous mice were stained with anti-CD127-biotin/streptavidin-allophycocyanin and either anti-CD3-PE or anti-CD61-PE and then were stained with 7-AAMD and analyzed with a FACSCalibur. *A*, Side scatter vs forward scatter of the cells analyzed. Region 1 (R1), including lymphocytes and smaller size megakaryocyte, and R2, including larger size megakaryocytes (41), in the 7-AAMD-negative live cells are indicated in the dot plot. *B*, R3, including CD127$^+$ cells, and R4, including CD61$^+$ cells in R1, and R5, including CD61$^+$ cells in R2, are indicated in the contour plot. *C*, Expression of GFP as analyzed by gating R3–R5 is shown in the histogram plot. The staining obtained in Tg mice is represented by the bold line, and the staining in wild-type mice is represented by the thin line.

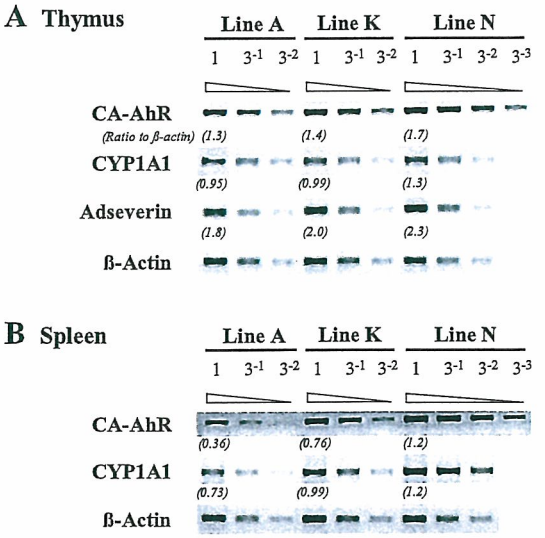

FIGURE 4. Comparison of CA-AhR and target gene expression in the thymus and spleen from line A, line K, and line N heterozygous mice. Total RNA was prepared from the thymus and spleen of the three lines with the RNeasy mini kit. cDNAs prepared from 20 ng of total RNA and serial dilutions (3^{-1}–3^{-3}) were amplified by PCR using primers for CA-AhR, CYP1A1, adseverin, or β-actin as a housekeeping gene. The expression of genes was quantified by densitometrically scanning gel images, and the values normalized to β-actin mRNA are indicated in parentheses. The numbers of PCR cycles for CA-AhR in thymus and spleen were 32 and 34, respectively, 26 and 32 for CYP1A1, and 20 for β-actin in both tissues. Mice were used after crossing into C57BL/6 mice for six generations in line A and for three generations in lines K and N.

CA-AhR expression induces target gene mRNA in both thymus and spleen, but reduces thymocyte number alone

Expression of the CA-AhR transgene and its target genes and phenotypic changes in the thymus and spleen were examined in the three lines. RT-PCR analyses showed that CA-AhR and CYP1A1 expression in the thymuses and spleens of lines A, K, and N were increased according to the integrated CA-AhR gene numbers (Fig. 4). Expression of adseverin, which was reported to be induced by TCDD in mice thymuses in an AhR-dependent manner (37), was also increased according to the transgene numbers.

The thymus weight was reduced in all three lines, by 36% in line A, 70% in line K, and 63% in line N (Fig. 5*A*). The thymocyte number was reduced by 49% in line A, 96% in line K, and 92% in line N (Fig. 5*A*). The thymocyte population defined by CD4 and CD8 expression was also affected in the Tg mice, with reduced percentages of CD4$^+$CD8$^+$ DP cells and increased percentages of CD8 SP and double negative (DN) cells (Table II). The ratios of CD4 SP/CD8 SP were significantly reduced in the Tg mice (Table II). The remarkable increases in the percentage of DN cells in line K and N were parallel to the large decreases in the total cell numbers.

By contrast, spleen weight was unaffected by the expression of CA-AhR (Fig. 5*B*). Splenocyte number was significantly reduced only in line N (by 40%), in which CA-AhR is most highly expressed (Fig. 5*B*), and the percentages of CD4 T cells, CD8 T cells, and B cells were unchanged, even in line N (data not shown). All of these findings are consistent with those observed in the thymuses exposed to TCDD (18, 21, 22).

We further confirmed that the CA-AhR is not expressed in the stromal cells and that CA-AhR expression in T-lineage cells alone is capable of inducing the thymus alteration in FTOC. mRNA expression was examined in whole thymus and stroma obtained by culturing thymus tissues in the presence of 2-deoxyguanosine to deplete it of thymocytes. As expected, CD4 mRNA was detected only in whole thymus, and Spatial mRNA, which is specifically expressed in thymic stromal cells (42), was detected in both the

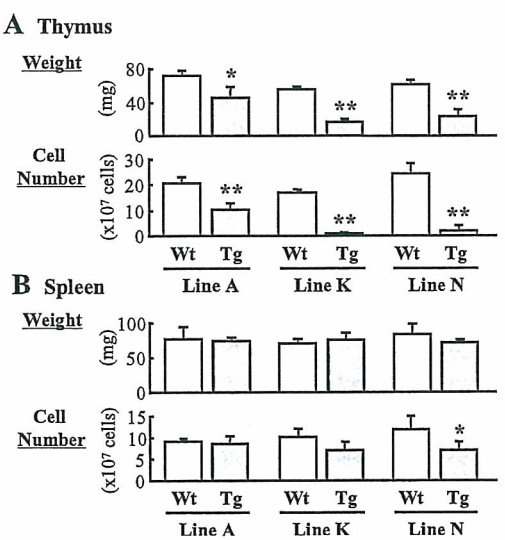

FIGURE 5. CA-AhR expression in T-lineage cells reduces thymus weight and cell number, but affects the spleen less. Thymus and spleen from female heterozygous Tg mice and nontransgenic littermate wild-type mice of line A (8 wk old, n = 4/each group), line K (10 wk old, n = 5), and line N (8–9 wk old, n = 5) were examined. Mice were used after crossing into C57BL/6 mice for two generations in line A and for three generations in lines K and N. The differences between Tg mice and wild-type mice were analyzed by Student's t test. The data are expressed as mean ± SD. *, $p < 0.05$; **, $p < 0.01$.

Table II. *CA-AhR expression affects thymocyte population*[a]

	Line A (n = 4)		Line K (n = 5)		Line N (n = 5)	
	Wt	Tg	Wt	Tg	Wt	Tg
	(%)		(%)		(%)	
DN	4.7 ± 0.5[b]	6.4 ± 1.2	4.2 ± 1.1	52.6 ± 7.8	6.8 ± 2.8	27.9 ± 14.1
DP	82.2 ± 1.7	79.3 ± 1.4	87.2 ± 1.8	33.5 ± 7.6	83.2 ± 2.4	53.1 ± 15.9
CD4 SP	10.0 ± 1.5	9.5 ± 2.0	6.9 ± 0.7	8.7 ± 1.2	7.0 ± 0.8	10.2 ± 0.9
CD8 SP	3.1 ± 0.2	4.9 ± 0.4	1.8 ± 0.4	5.2 ± 0.5	3.1 ± 0.3	8.8 ± 1.2
CD4/CD8[c]	2.6 ± 1.1	2.0 ± 0.5	4.0 ± 0.7	1.8 ± 0.6**	2.3 ± 0.3	1.2 ± 0.1**

[a] Thymocytes from female heterozygous (CA-AhR$^{+/-}$) Tg mice and nontransgenic (CA-AhR$^{-/-}$) littermate mice (Wt) (8–10 wk old) were examined by flow cytometry.
[b] The data was expressed as means ± S.D. The differences between CD4/CD8 ratio in Tg mice and Wt mice were evaluated with Wilcoxon rank sum test. **, $p < 0.01$.
[c] Ratio of CD4 SP cells/CD8 SP cells.

whole thymus and the stroma (Fig. 6). CA-AhR was detected only in whole thymus, not in the stroma, in this system (Fig. 6). Thus, AhR activation in thymocytes alone was demonstrated to cause the cellular loss and population changes in the thymus.

CA-AhR suppresses the increase in spleen weight and splenocyte number caused by immunization

We previously reported finding that TCDD administration to mice immunized with OVA suppressed the immunization-induced increase in spleen weight and splenocyte number (27, 34). Consistent with these findings, the increase in spleen weight and splenocyte number observed in wild-type mice after immunization with OVA was suppressed in line A Tg mice (Fig. 7), although their spleen was unaffected when not immunized, as stated above (Fig. 5). Interestingly, increases of both CD4 T cells and B cells were significantly suppressed despite the specific expression of CA-AhR in T cells (Fig. 7). The number of CD8 T cells was also fewer in the Tg mice than in the wild-type mice, although the difference was not significant. Simultaneous suppression of the T and B cell increase was also observed in OVA-immunized and TCDD-exposed wild-type mice (27).

To estimate how much TCDD induces the corresponding level of AhR activation, CYP1A1 expression in the thymus and spleen of line A Tg mice and TCDD-exposed wild-type mice was compared. As shown in Fig. 8, the level of expression of CYP1A1 mRNA in the thymus of the Tg mice was slightly higher than its level of expression in wild-type mice exposed to a single dose of 20 μg/kg TCDD. CYP1A1 expression in the spleen of the Tg mice was less than in wild-type mice exposed to 20 μg/kg TCDD, which seems plausible because only T cells express CYP1A1 mRNA in the spleen of Tg mice, whereas both T and B cells express CYP1A1 mRNA in TCDD-exposed wild-type mice (43). These results show that the function of the activated AhR in line A heterozygous mice and AhR activated by 20 μg/kg TCDD are roughly comparable. In our previous study, 20 μg/kg TCDD suppressed the splenocyte number to 60% of the number in unexposed control mice 1 wk after immunization (27). The same dose of TCDD reduced the thymus weights to 40–60% (44). The fact that a similar extent of suppression was observed in the thymus and spleen of the Tg mice indicates that the major portion of the effect of TCDD in these organs is attributable to AhR activation in the T cells.

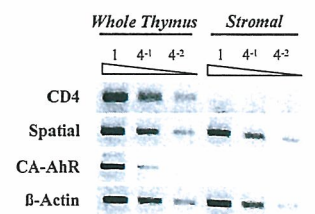

FIGURE 6. CA-AhR is expressed in the thymocytes, but not in the stromal cells, in the thymus. line A male CA-AhR$^{+/+}$ mice were mated with female C57BL/6J mice, and thymuses were collected from fetuses on gestational day 16.5. The thymuses were cultured for 4 days on a nitrocellulose filter floated on complete medium. To deplete them of thymocytes and obtain stromal cells, the lobes were cultured in the presence of 1.35 mM 2-deoxyguanosine. Fifteen or 16 lobes were pooled from each treatment group and were used to prepare RNA. cDNAs prepared from 20 ng of total RNA and serial dilutions (4^{-1}, 4^{-2}) were amplified by PCR

Discussion

To prove the role of activated AhR in T cells in TCDD-induced immune suppression, we generated Tg mice in which expression of CA-AhR cDNA is controlled by an improved version of hCD2 promoter and their T cells specifically express activated form of AhR. We obtained three lines bearing different copy numbers of the transgene, ranging from 2 to 10. All three lines showed expression of CA-AhR and an AhR-induced target gene in the thymus and spleen. In line A mice, which coexpress GFP in addition to CA-AhR under the control of the same CD2 promoter, the transgene was confirmed to be strongly expressed in the thymocytes, CD4, and CD8 T cells, but not in the thymus stromal cells or in the B cells. CD2 is principally expressed on T cells and NK cells in humans (45), but low expression has also been reported on subsets of other cell types, including B cell progenitors (46, 47). Consistent with the observation, faint expression of the transgene was detected on the CD3$^-$CD127$^+$ lymphocyte progenitor cells in our

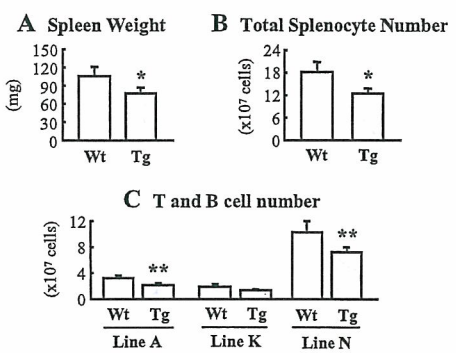

FIGURE 7. CA-AhR expression suppresses the increase in spleen weight and both CD4 T cell and B cell numbers after immunization. Line A female heterozygous Tg mice and littermate wild-type mice (8 wk old, n = 4) were immunized with OVA/alum, and their spleens were examined 7 days later. Mice were used after crossing into C57BL/6 mice for five generations. The differences between the Tg mice and wild-type mice were analyzed by Student's t test. The data are expressed as mean ± SD. *, $p < 0.05$; **, $p < 0.01$.

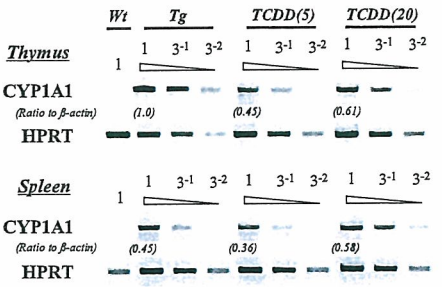

FIGURE 8. Comparison of CYP1A1 induction by CA-AhR in Tg mice and by TCDD exposure. Female C57BL/6 mice were given 5 or 20 μg/kg TCDD, and 3 days later total RNAs were prepared from thymocytes and splenocytes. CYP1A1 induction was compared with its induction in female line A heterozygous mice. cDNAs prepared from 20 ng of total RNA and serial dilutions (3^{-1}, 3^{-2}) were amplified by PCR using primers for CA-AhR or hypoxanthine phosphoribosyltransferase as a housekeeping gene. The expression of genes was quantified by densitometrically scanning gel images, and the values normalized to β-actin mRNA are indicated in parentheses. In both tissues, 30 PCR cycles were used for CYP1A1 and 20 for hypoxanthine phosphoribosyltransferase.

Tg mice. The transgene was also found to be expressed in the lung and, to a very minor extent, in the kidney in the Tg mice. Although the mechanism for the expression of CA-AhR in those tissues is unknown, presence of cells expressing CA-AhR in those tissues, such as T cells in the lung, may partly contribute to the transgene expression. Albeit the expression of the vector is found in other cell types, these CD2-based vectors, including the VA hCD2 vector, have been proved to be very useful to study specific functions of molecules in T cells. Likewise, the Tg mice we developed in the present study enable a new approach to explore the effect of AhR activation in T cells in the immune suppression. Lymphocyte progenitor cells have been reported to be affected by TCDD, and its effect was suggested to contribute to a loss of thymocytes (48, 49). Although the expression of CA-AhR in the lymphocyte progenitor cells was much fainter than that in the thymocytes and T cells (Figs. 2 and 3), the effects of low expression of CA-AhR may need to be considered.

All three lines of our Tg mice were characterized by thymus involution, including reduced thymocyte number and increased percentage of CD8 SP cells, the same as observed in TCDD-exposed mice. The fact that direct exposure of FTOC to TCDD reproduces the thymus involution induced by TCDD exposure in vivo (19, 24) shows that TCDD directly affects the thymus, in which the target cells are present. However, the results of previous studies have suggested that two types of cells in the thymus, thymocytes (18, 28) and stromal cells (24, 25), are the primary targets. The results of the present study in the Tg mice demonstrate that AhR activation in T-lineage cells alone can cause the thymus alterations, including loss of thymocytes and increase in percentage of CD8 SP thymocytes, without AhR activation in the stromal cells. Tomita et al. (50) recently produced T cell-specific ARNT-deficient mice in which the ARNT gene is disrupted under the control of T cell-specific $p56^{lck}$ proximal promoter, and showed that the thymus of the Tg mice is resistant to TCDD. Their results are consistent with our own showing that the AhR/ARNT heterodimer in the thymocytes, but not stromal cells, is essential for the occurrence of thymus involution.

Whereas thymus undergoes involution upon TCDD exposure, the splenocytes and splenic T cells of nonimmunized animals are unaffected by TCDD (18, 51). The same finding was observed in the spleen of our T cell-specific CA-AhR Tg mice, even though the CA-AhR was fully expressed in both the spleen T cells and the thymocytes. Although we examined the expression of CYP1A1 and adseverin as sensitive AhR-dependent target genes to estimate the extent of AhR activation, the genes responsible for the thymus involution remain to be identified. Previous studies have suggested suppression of thymocyte proliferation (24, 28) and induction of apoptosis (52) as the biological process involved in the thymus atrophy caused by TCDD. Our own recent study demonstrated that CA-AhR expression in Jurkat T cells inhibits cell growth by inducing both apoptosis and cell cycle arrest (29). Several genes in these CA-AhR-expressing Jurkat T cells that are related to apoptosis or cell cycle arrest, such as Fas, cyclin G_2, and growth arrest and DNA damage-inducible protein 34, were shown to be up-regulated in an XRE-mediated transcription-dependent manner (29), and these genes may be responsible for the loss of thymocytes.

In contrast with the nonimmunized mice in which splenocytes were less affected by AhR activation, as described above, the increase in splenocyte number after immunization was suppressed in the CA-AhR Tg mice, suggesting that the AhR/ARNT heterodimer inhibits cell growth in activated and proliferating T cells, but not in resting T cells. From this point of view, the effect of AhR activation in thymocytes may be also attributable to the effect on activated or proliferating cells: in thymus atrophy, the suppression of DN cell proliferation (28) and the loss of DP cells (52) are suggested to be responsible for cellular loss, DN thymocytes are vigorously proliferating cells, and DP cells receive an activation signal via their T cell receptors. Thus, activation state of the cell seems to affect the sensitivity of T-lineage cells to AhR activation.

The results of the present study also demonstrated that AhR activation in T cells alone suppresses the increase in both T and B cells in the spleen after OVA immunization. In terms of primary target cells of TCDD toxicity in immune reaction, Kerkvliet et al. (26) recently showed that AhR in both CD4 and CD8 T cells is necessary for full suppression of CTL response by TCDD in a mouse acute graft-vs-host model in which T cells, or CD4 or CD8 subsets, from $AhR^{+/+}$ and $AhR^{-/-}$ C57BL/6 mice were injected into C57BL/6 × DBA/2 F_1 host mice. Consistently, our results indicated that AhR activation in T cells is involved in changes in immune reaction. We previously reported that TCDD administration to OVA-immunized mice suppresses the growth of T and B cells and the production of Th2-type cytokines before suppression of Ab production (27, 34), which suggested that TCDD inhibits Ab production by suppressing T cell activation and the subsequent Th2-cell differentiation. The results of the present study strongly support the hypothesis that activation of the AhR directly inhibits cellular activation of the T cells and their subsequent proliferation and differentiation, leading to the suppression of T cell help on B cell proliferation. Alternatively, indirect effect of $CD4^+CD25^+$ regulatory T cells may be involved in the immune suppression. The regulatory T cells are positively selected in the thymus when their TCR receives a signal with intermediate strength (53). TCDD exposure to thymus is shown to affect thymocyte selection, possibly through up-regulation of Notch 1 (54) or activation of the ERK pathway (35) in the thymocytes, and these mechanism may alter the selection of $CD4^+CD25^+$ regulatory T cells. Further study of our Tg mice will clarify whether the suppression of T cell growth by AhR activation inhibits Th2-type cytokines and following Ab production by B cells, and it will also provide a clue for solving the mechanism of inhibition.

Andersson et al. (55) recently produced Tg mice expressing a CA-AhR mutant (30), which has a structure very similar to the one we used, under the control of an Ig H chain enhancer ($E\mu$), which promotes transgene expression in both B- and T-lineage cells (56). In addition to exhibiting thymus atrophy, their mice have a reduced life span and develop tumors in the glandular part of the stomach

(55). The Tg mice generated in our study, in contrast, are fertile and do not exhibit any overt phenotype differences except thymus atrophy, showing that AhR activation in T cells is not responsible for the stomach tumors.

Although T cells contain functional AhR and directly respond to TCDD (57), phenotypic changes caused by TCDD in T cells, such as changes in proliferation and differentiation, are difficult to detect in vitro, possibly because of optimized culture conditions that compensate for the effects of TCDD (6). The Tg mice expressing CA-AhR in T cells will be a useful model for investigating the role of activated AhR in the T cells. In particular, immunization of the Tg mice is expected to show suppression of various immune reactions, including Ab production and CTL activity, the same as observed in TCDD-exposed mice. Dioxins are persistent environmental contaminants and as such animals are continually exposed to them. TCDD maternally exposed is transferred to fetus and pups through the placenta or milk and activates their AhR (58, 59). Our Tg mice express CA-AhR mRNA in the fetal thymuses (data not shown) and the expression continues in the T-lineage cells after birth. Thus, these Tg mice will also be a useful model for clarifying the effect of persistent activation of AhR in T cells. Studies using our CA-AhR Tg mice should shed light on the role of the AhR in T cells in immune suppression by TCDD and also in physiological reactions.

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Disclosures

The authors have no financial conflict of interest.

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