# REDUCED SUSCEPTIBILITY TO IL-1 AND ENDOTOXIN IN TRANSGENIC MICE EXPRESSING IL-1 IN THEIR LENS

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**To learn about the effects of chronic exposure to IL-1 we generated a transgenic (Tg) mouse line that expresses human IL-1β under the control of the lens αA crystallin promoter. Expression of human IL-1β was restricted to the eye; neither the protein nor its mRNA were detected in various other organs of the Tg mice. The Tg mice develop severe ocular inflammation shortly after birth, which affects the lens and other eye tissues and apparently allows the release of IL-1 into the circulation. Here we report that the Tg mice exhibit decreased responsiveness to IL-1 and lipopolysaccharide (LPS), as compared to their wild-type littermate controls: (1) when injected with IL-1 the Tg mice produced lower levels of serum amyloid A than their controls; (2) thymocytes of the Tg mice responded less vigorously in culture to stimulation with IL-1; and (3) Tg mice showed lower morbidity and mortality than their controls when injected with toxic amounts of LPS. These data suggest that chronic exposure to IL-1 in the Tg mice induces partial resistance to this cytokine, analogous to the reduced responsiveness to IL-1 in animals pretreated with this proinflammatory cytokine.**



Interleukin 1 (IL-1) is a pro-inflammatory cytokine involved in many of the acute and chronic inflammatory processes of numerous diseases (reviewed in Refs 1–4). Among its many functions, IL-1 acts locally in the recruitment of inflammatory cells by upregulating the expression of cell adhesion molecules and other proinflammatory cytokines such as IL-6, IL-8, or tumour necrosis factor alpha (TNF-α). Systemically, IL-1 acts as an endogenous pyrogen, induces the acute phase response and upregulates the production of vasodilatory compounds such as platelet activating factors, prostaglandins and nitric oxide. Understanding of the local and systemic effects of IL-1 has been mainly gained from experimental animal models in which IL-1 has been artificially elevated for limited durations.[1–4] We have recently succeeded in generating the first transgenic (Tg) mouse in which IL-1 is produced continuously, by placing the human IL-1β gene under the control of the lens-specific mouse αA-crystallin promoter. These Tg mice developed progressive severe ocular inflammation that culminated in phthisis bulbi.[5] The present study analysed the responsiveness of the Tg mice to IL-1 or to stimuli in which IL-1 plays an active role. The Tg mice exhibited reduced responses to all tested stimuli when compared to their wildtype (WT) littermate controls.

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## RESULTS

### *Human IL-1β in Tg mice*

Human IL-1β was detected in eye extracts from Tg mice at all ages tested. Figure 1 records the IL-1 concentrations, presented as normalized values of ng/mg eye protein (A), or as ng/eye (B). The cytokine levels were found to decline markedly with age during the period covered by this study, with the values being highest at 6 weeks and lowest at 24 weeks. It is noteworthy that eyes of aged mice were also smaller in size, due to the damaging inflammatory process.[5] Human IL-1β, however, was not detected in the eyes of WT mice, nor in the blood, thymus, spleen, liver or heart of the Tg mice, using the commercial ELISA kit, with the sensitivity threshold of 4 pg/ml (data not shown). In addition, human IL-1β mRNA was detected by Northern blot

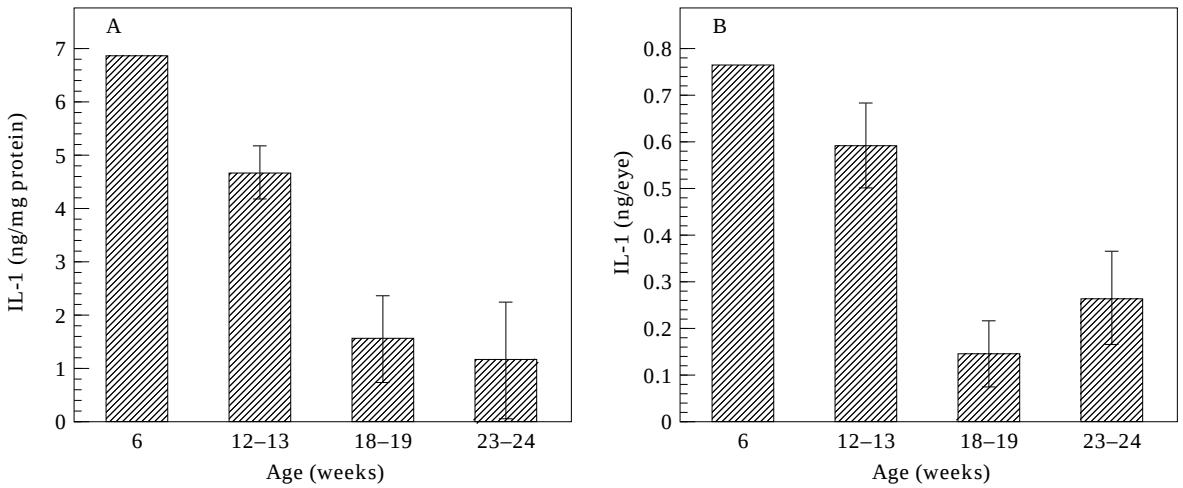


**Figure 1. Levels of human IL-1 in eyes of Tg mice at different ages.**

Eye extracts were prepared and IL-1 concentrations were measured as described in Materials and Methods. Data are presented as ng/mg protein (A), or ng/eye (B).

analysis in 0.5 or 5 μg RNA samples of eyes from the Tg mice, but not in 20 μg RNA samples from 13 other organs of these mice (Fig. 2).

### *Lowered production of IL-1-stimulated serum amyloid A (SAA) in the Tg mice*

SAA levels in plasma of untreated Tg mice were similar to those of their WT controls; the mean values (± S.E.) from repeated assays, with a total of seven mice in each group, were 21.8 ± 2.4 and 17.0 ± 4.3 μg/ml, respectively. When challenged with IL-1, however, the Tg mice responded significantly less than their controls by producing lower levels of SAA (Fig. 3). This decreased response of Tg mice was found with groups of mice injected with IL-1 at either 1000 (Fig. 3A) or 100 ng (Fig. 3B).

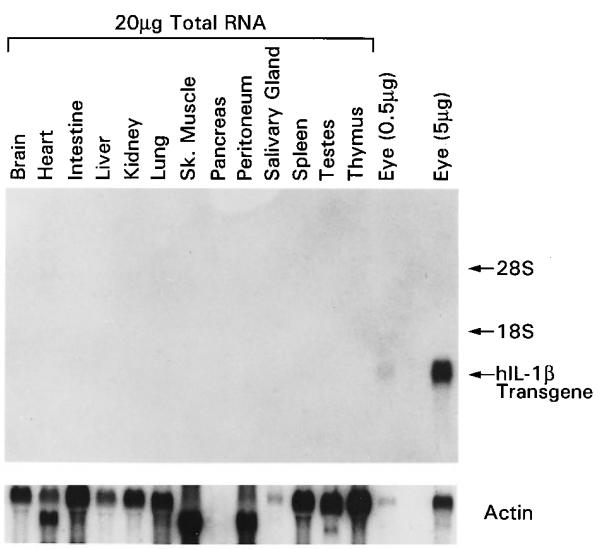


**Figure 2. Northern blot analysis showing mRNA of human IL-1β in the eyes but not in various other tissues of Tg mice.**

The amount of total RNA used for each sample is indicated on the Figure and the procedure is detailed in Materials and Methods. The size of the hybridizing band (~950 nt) is consistent with that expected from the transgene (865 nt + poly A tract) and is significantly smaller than the endogenous murine IL-1β mRNA (1339 nt + poly A tract).

### *Lowered responsiveness to IL-1 by thymocytes of Tg mice*

Thymocytes from Tg mice responded to IL-1 less vigorously than thymocytes from the WT controls (Fig. 4). The reduced responsiveness was observed in cultures stimulated with either IL-1 alone or in combination with phytohemagglutinin (PHA). Different batches of IL-1 were used in these experiments, producing variability in the levels of proliferation. The lowered response of the Tg mice was particularly pronounced in mice 8–9 weeks old, less in the 6–7 weeks old mice and least in the 14–16 weeks old group. Unlike their lowered thymocyte response to IL-1, the Tg mice resembled and even slightly exceeded their littermate WT controls in their thymus cell contents at all tested ages (legend for Fig. 4).

### *Lowered susceptibility of the Tg mice to the toxic effect of lipopolysaccharide (LPS)*

Morbidity and mortality induced by LPS injection were remarkably less pronounced in the Tg mice than in the WT controls (Fig. 5). All 21 WT mice became sick within 6 h post-injection and 18 (86%) of them died by the 72 h time point. On the other hand, only 50% of the Tg mice showed the signs of sickness, at later time points than the WT mice, and only 6 of the 16 (37%) animals of this group died by 72 h.

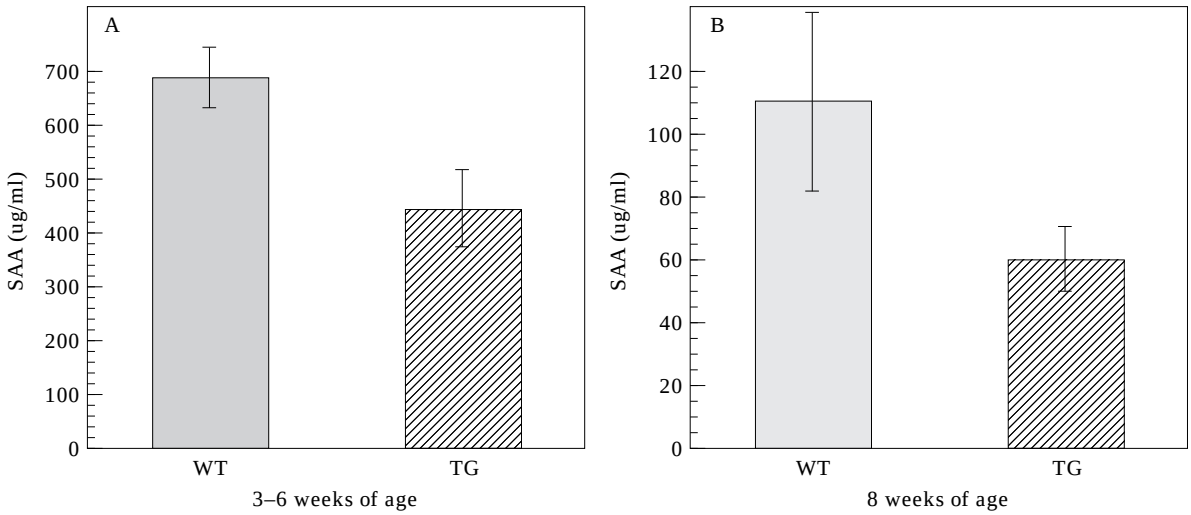


**Figure 3. SAA production is lower in Tg mice than in their WT littermate controls.**

Groups of mice were injected with human IL-1α at 1000 ng/mouse (A) or 100 ng/mouse (B). Mice used in A were 3–6 weeks old, while those in B were 8 weeks old. The difference between the SAA levels in Tg and WT mice was statistically significant ($P = 0.05$, by the unpaired *t*-test) in experiment A, but insignificant in experiment B.

## DISCUSSION

The present study has provided for the first time information concerning the immunological profile of mice in which IL-1 is continually expressed due to a transgenic manipulation. The Tg mice used in the present study were all of a single line; repeated attempts to create additional lines have all failed, presumably because of the lethal effects of this potent cytokine. The one line which survived was demonstrated by Southern blots to have only a single copy insertion of the transgene.[5] It is relatively rare to find single copy insertions of a transgene and, perhaps, this fortuitous event allowed for the survival of this line of Tg mice. It is also of interest that a similar observation was made with Tg mice expressing another cytokine, IL-2; only mice with a single copy of the transgene were found to survive.[6]

Although no IL-1 could be detected in serum samples of the Tg mice by the available assays, it is assumed that the cytokine was released into the circulation of these animals as early as day 12 of gestation, when the αA–crystallin promoter is turned on.[7] Eyes of the Tg mice were severely damaged,[5] disrupting the natural sequestration of this organ. Moreover, changes in the immunological behaviour of these animals suggest that IL-1 was continually released and was the cause for these changes. The decrease in responsiveness to IL-1 stimuli was demonstrated both in vivo and ex vivo. In the in vivo assay, Tg mice were found to produce less SAA than their WT littermate controls when challenged with IL-1 (Fig. 3). SAA is a plasma protein produced by the liver during the acute phase response to injury or infection[8–10] and its synthesis is upregulated predominantly by IL-1 and IL-6.[8–12]

The reduced responsiveness to IL-1 was shown ex vivo by comparing the proliferative reaction to this cytokine of thymocytes from Tg and WT mice. Tg thymocyte cultures responded less vigorously than their WT control cultures when incubated with IL-1, alone or in combination with PHA (Fig. 4). It is of interest that the difference between Tg and WT mice was found to diminish with age, possibly due to the reduced levels of IL-1 in aged Tg mice (Fig. 1).

In addition to their reduced response to IL-1, Tg mice exhibited lowered susceptibility to the toxic effects of LPS, i.e., injection of this bacterial product caused much less sickness and death in the Tg mice than in their WT controls (Fig. 5). The involvement of IL-1 in the LPS toxic effect was indicated in several studies;[2,13–15] this cytokine is assumed to play a major role in the LPS pathogenic process, along with other cytokines, particularly TNF-α and IL-6.[16–18]

The reduced susceptibility to IL-1-mediated processes in the Tg mice in the present study is in line with the observations that pretreatment of rats with low levels of IL-1 reduced the severity of antigen-induced arthritis,[19] or elicited desensitization against the lethal effect of this cytokine.[20] Moreover, pretreatment with IL-1[21–23] or other proinflammatory cytokines[24] confers resistance to lethal infection. It is also noteworthy that a state of resistance to LPS was induced in mice by treatment with a cytokine-inducing agent, complete Freund's adjuvant.[25]

Our observations with the IL-1 Tg mice are also of interest in view of the recent finding that mice deficient in IL-1β converting enzyme (ICE) are partially resistant to the toxic effects of LPS.[14] It is thus remarkable that genetically created chronic exposure to IL-1 produces a phenotype similar to that of IL-1 deficiency in its resistance to LPS.

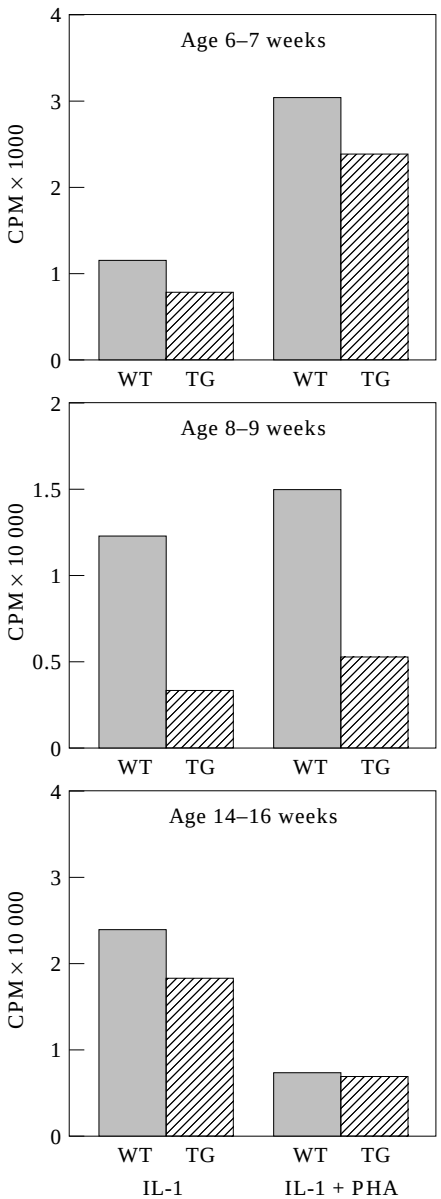


**Figure 4. Reduced responsiveness to IL-1 by thymocytes from Tg mice.**

Proliferative responses to IL-1, alone or in combination with PHA, were carried out with thymocytes from Tg mice and their littermate WT controls at the indicated ages. Thymi from two or three Tg and WT mice were individually tested at each age point and the data are presented as the mean cpm values of stimulated cultures of all mice of each group. The mean cell numbers ($\times 10^6$/thymus) obtained from thymi of WT and Tg mice were 79 and 90 at 6–7 weeks of age, 55 and 62 at 8–9 weeks and 43 and 51 at 14–16 weeks, respectively.

The mechanism(s) whereby the Tg mice acquire the reduced susceptibility to IL-1 is not clear. Analysis of the resistance to infection in mice pretreated with IL-1 has shown that exposure to this cytokine initiates several mechanisms capable of inhibiting the pathogenic processes mediated by proinflammatory cytokines. These mechanisms mainly include downregulation of the proinflammatory cytokines and their receptors on

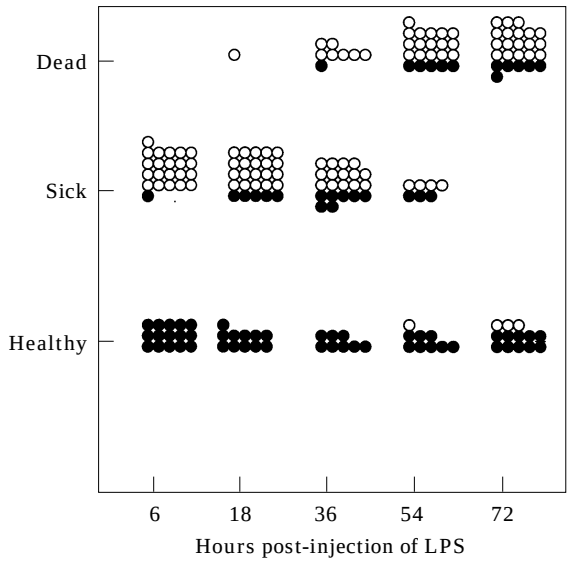


**Figure 5. Morbidity and mortality in Tg mice (●) and their WT controls (○) at different time points following the injection of LPS.**

Closed circles represent individual Tg mice and open circles, the WT controls. The difference in mortality at 72 h between the Tg and WT groups was statistically highly significant ($P = 0.002$, by the chi square test).

cells throughout the body,[2,23,26] as well as upregulation of IL-1 receptor antagonist (IL-1ra).[2,23,27] It is conceivable that these mechanisms are also activated by the chronic release of IL-1 in the Tg mice and are responsible for the reduced responsiveness to IL-1 and LPS in these animals. This hypothesis is currently under investigation.

## MATERIALS AND METHODS

### *Construction of the IL-1β vector*

The human IL-1β expression cassette, containing sequences encoding the human tissue plasminogen activator secretion signal peptide fused in frame to the coding region of mature human IL-1β and the bovine growth hormone polyadenylation signal, was excised with Kpn I and Eco47 III from plasmid TPAIL-1β mature.[28] This fragment was put downstream of the murine αA-crystallin promoter to form pEW34. The transgene was excised from the plasmid by digestion with Sal I and Not I and gel purified in preparation for pronuclear microinjection.

### *Creation of Tg mice*

Standard methods were used to generate Tg mice by injecting the transgene DNA (4 μg/ml) into a pronucleus of single celled mouse embryos of the FVB/N strain. Mouse lines were established by mating founder animals to normal FVB/N mice. For the present study, Tg FVB/N mice were mated to normal DBA2 mice and the resulting (FVB/N × DBA2) F1 hybrid mice were used throughout this study. WT littermates were used as control animals.

### *Quantification of IL-1*

Levels of human IL-1β were measured in extracts of eyes or other organs, as well as in sera of Tg mice and WT littermate controls ranging from 6 to 24 weeks of age. Tissue extracts were prepared by homogenizing the organs in ice cold phosphate buffered saline, centrifuging the homogenates at 500 × ***g*** for 30 min and collecting the supernatants. Levels of human IL-1β were measured using a commercial ELISA kit (Cistron Biotechnology, Pine Brook, NJ). Protein concentrations in the extracts were determined by the BCA Protein Assay Kit (Pierce, Rockford, IL).

### *Northern blot analysis*

Total RNA was isolated from tissues of a 30 day old male transgenic mouse by the RNAzol B Method (Cinna/Biotecx Laboratories, Friendswood, TX) according to the manufacturer's instructions. The RNA was fractionated in a 1.5% agarose gel containing 6.7% formaldehyde and 1 × MAE (20 mM MOPS [pH 7.4]; 5 mM sodium acetate; 0.5 mM EDTA). For most tissues, 20 μg of total RNA was used, but for the eye 5 and 0.5 μg of RNA were used. Following electrophoresis, the RNA was blotted onto a nitrocellulose membrane (Schliecher & Schuell, Keene, NH) with 20× SSC (1 × SSC contains 0.15 M NaCl; 0.015 M sodium citrate) and UV crosslinked to the membrane. Before blotting, lanes containing RNA molecular weight standards were cut from the gel and stained with ethidium bromide. The blot was prehybridized in 5× SSPE (1× SSPE contains 0.01 M $NaPO_4$ [pH 7.4]; 0.15 M NaCl; 0.001 M EDTA); 50% formamide; 5× Denhardt's solution; 0.1% SDS; 0.1 mg/ml denatured salmon sperm DNA at 42°C for 3 h. It was then hybridized overnight at 42°C in the same solution containing $^{32}$P-labelled probe derived from the human IL-1β portion of the transgene. Conditions for the final wash of the blots were 55°C in 0.1 × SSPE; 0.2% SDS. After autoradiography, the blot was reprobed with a human β-actin probe to determine RNA integrity.

### *Quantification of SAA*

Levels of SAA in the plasma of Tg and WT control mice were measured without treatment or 14–16 h following injection of recombinant human IL-1α (a generous gift from Dr Peter Lomedico, Hoffmann-La Roche), at 100 or 1000 ng/mouse. Plasma samples were obtained by collecting the mouse blood into EDTA-lined tubes and the levels of SAA were determined using a modification of a direct binding ELISA[29] in which plates were coated with the plasma samples for 2 h at 37°C. The blocking step was omitted and following washing with $H_2O$, wells were incubated with polyclonal rabbit antiserum against synthetic peptides corresponding to residues 94–103 of mouse apo $SAA_1$ and apo $SAA_2$.

### *Thymocyte proliferation assays*

Thymocyte proliferation assays[30] were set up in triplicate in 96 well flat bottom plates. Thymocytes ($1 \times 10^6$ cells/well) were cultured in a final volume of 0.2 ml RPMI-1640 medium (Mediatech, Herndon, VA) supplemented with 5% fetal calf serum (HyClone, Logan, Utah), L-glutamine (2 mM), penicillin (100 U/ml) and streptomycin (100 μg/ml). Cells were stimulated with recombinant human IL-1α alone at a final concentration of 10 ng/ml, or at 0.1 ng/ml in combination with PHA (Murex Diagnostics, Dartford, UK, at 1 μg/ml). After incubation for 48 h at 37°C in a humidified atmosphere of 5% $CO_2$, cultures were pulsed for 16 hrs with $^3$H-thymidine, 2 Ci/mmol, 0.5 μCi/10 μl/well. Cultures were then harvested and the radioactivity on the collecting filters was measured by scintillation counting. The data are presented as mean cpm values of the stimulated cultures (with IL-1 alone, or IL-1+ PHA) of all mice of each group.

### *Susceptibility to endotoxin effects*

A total of 21 WT and 16 Tg mice, 6–8 weeks old, were injected intraperitoneally with LPS serotype 0127:B8 (Sigma, St. Louis, MO) at a final dose of 40 μg/gram body weight. Animals were then observed every 6 h for 72 h and rated as either healthy, sick or dead. Sick mice were lethargic and exhibited raised fur and occasional shivering.

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