Cell Death and Differentiation (2006) 13, 861–872

www.nature.com/cdd

Review

Dissection of the NF-κB signalling cascade in transgenic and knockout mice

M Pasparakis*,[1,2], T Luedde[1] and M Schmidt-Supprian[3]

[1] European Molecular Biology Laboratory (EMBL), Mouse Biology Unit, Via Ramarini 32, Monterotondo-Scalo, Rome 00016, Italy
[2] Institute for Genetics, University of Cologne, Zülpicher Str. 47, Cologne, Germany
[3] The CBR Institute for Biomedical Research, Harvard Medical School, 200 Longwood Avenue, Boston, MA, USA
* Corresponding author: M Pasparakis, European Molecular Biology Laboratory (EMBL), Mouse Biology Unit, Via Ramarini 32, Monterotondo-Scalo, Rome 00016, Italy. Tel: +39 06 90091 222; Fax: +39 06 90091 272; E-mail: pasparakis@embl-monterotondo.it

Received 02.11.05; revised 20.12.05; accepted 23.12.05; published online 10.2.06
Edited by G Kroemer

Abstract

Studies in transgenic and knockout mice have made a major contribution to our current understanding of the physiological functions of the NF-κB signalling cascade. The generation and analysis of mice with targeted modifications of individual components of the NF-κB pathway tremendously advanced our knowledge of the roles of the NF-κB proteins themselves, and also of the many activators and negative regulators of NF-κB. These studies have highlighted the complexity of the NF-κB system, by revealing the multiple interactions, redundancies, but also diverse functions, performed by the different molecules participating in the regulation of NF-κB signalling. Furthermore, inhibition or enforced activation of NF-κB in transgenic mice has uncovered the critical roles that NF-κB plays in the pathogenesis of various diseases such as liver failure, diabetes and cancer.

Cell Death and Differentiation (2006) **13,** 861–872.
doi:10.1038/sj.cdd.4401870; published online 10 February 2006

Keywords: gene targeting; NF-κB signalling; transgenic mice

Abbreviations: IKK, IκB kinase; ES cells, embryonic stem cells; IKK2CA, constitutively active mutant IKK2

The NF-κB Signalling Pathway

Among the numerous signalling cascades that are activated in response to extracellular stimuli and lead to transcriptional regulation, the NF-κB pathway is unique in its complex and rapid activation mechanism, the wide variety of inducing stimuli, and the large number of genes it regulates. The NF-κB signalling pathway functions in essentially all mammalian cell types and is activated in response to injury, infection, inflammation and other stressful conditions requiring rapid reprogramming of gene expression. In mammals, the NF-κB/Rel family of transcription factors consists of five members: p65/RelA, c-Rel, RelB, p50/p105 and p52/p100. NF-κB/Rel proteins form dimers that are kept inactive by association with inhibitory proteins belonging to the IκB family, which consists of IκBα, IκBβ, IκBε and Bcl-3, and also the p105 and p100 precursors of p50 and p52, respectively.

Activation of NF-κB is mediated by the IκB kinase (IKK) complex, which is composed of the IKK1/IKKα and IKK2/IKKβ catalytic subunits and the NEMO/IKKγ regulatory protein. Upon stimulation, the IKK complex phosphorylates IκB proteins on specific serine residues. This phosphorylation triggers a cascade of events, involving the polyubiquitination and proteasome-mediated degradation of IκB proteins, resulting in the nuclear accumulation of activated NF-κB dimers and induction of NF-κB-dependent gene transcription. Although degradation of IκB proteins remains a fundamental step for NF-κB activation, multiple mechanisms are implicated in the regulation of NF-κB transcriptional activity. Ubiquitination, sumoylation or phosphorylation of upstream and downstream mediators, IKK subunits or NF-κB proteins themselves is critical for the tight regulation of NF-κB signalling. Multiple enzymes control these processes and thereby regulate the activation of NF-κB. The large numbers of regulatory mechanisms that are in place to keep NF-κB activity under tight control allow NF-κB/Rel proteins to participate in numerous vital cellular functions.

Manipulation of NF-κB Signalling by Engineering the Mouse Genome

The dissection of the functions of the NF-κB pathway in physiological and pathological processes *in vivo* poses an unusual challenge, due to its complex mode of regulation and the functional redundancy and interplay between the different Rel proteins. Numerous studies applied transgenic technology in the mouse to study the function of NF-κB/Rel proteins, and also of activators and inhibitors of the NF-κB pathway, in an *in vivo* context. Indeed, nearly every technique available for the manipulation of the mouse genome has been employed for the analysis of the NF-κB signalling cascade, including the transgenic overexpression of activators or inhibitors of the pathway, conventional and conditional gene knockouts and also specific modifications of endogenous genes through targeted knock-ins (Table 1). In addition, the transgenic technology has been used to generate mice that allow monitoring of NF-κB activity *in vivo*, mostly by placing the expression of reporter transgenes under the control of NF-κB-responsive promoters (Table 1).

Here, we will attempt to provide examples for the contribution of transgenic studies to our current understanding of NF-κB function. Rather than presenting an extensive review of the relevant literature, our aim is to highlight the advantages

Table 1 Genetic mouse strains to study the nuclear factor-κB (NF-κB) pathway

Conditional alleles	
Ikk2^{FL}	17,18
Ikk2-ΔK^{FL}	24,27
Nemo^{FL}	6
IκBα^{FL}	99
C$^{loxP\text{-}I\kappa B\alpha\text{-}DN}$	51
Transgenic overexpression of IκB-DR	
T cells	35–38,54,80,81
B cells	68,69
Heart	47
Liver	40,49
Astrocytes	46
Airway epithelium	50
Muscle	48
Neurons	45
Pancreas	43,44
Skin	41,42
Transgenic overexpression of IKK-DN	
B cells	52
T cells	53
Neurons	28
Transgenic overexpression of IKK2CA	
Muscle	48
Liver	49
T cells	54
Neurons	28
Monitoring of NF-κB activity	
κB-β-globin	57
κB-EGFP	60
κB-LacZ	58,59
κB-luciferase	61–63
IκBα-luciferase fusion protein	64

A list of conventional knockout mice of the NF-κB pathway can be found at: http://people.bu.edu/gilmore/nf-kb/genek/index.html.

and potential shortcomings of each individual transgenic approach, by discussing the differences and discrepancies observed in the different studies.

NF-κB inhibition by transgenesis

Knockout of NF-κB/Rel proteins

The generation of 'knockout' mice using gene targeting in embryonic stem cells (ES cells) has been invaluable for the elucidation of gene function *in vivo* and has revolutionised biology over the last two decades. The application of knockout technology to the NF-κB pathway has had a major impact on our current understanding of the function of individual components of this signalling cascade. The ablation of Rel proteins has led to a range of different phenotypes highlighting the specific and redundant functions of individual NF-κB factors. The results obtained in these studies have been the subject of numerous comprehensive reviews (for example, see Li and Verma[1]), and will not be discussed here.

Conventional knockout of IKK subunits

The function of IKK subunits has also been studied in knockout mice: mice lacking NEMO or IKK2 die early during embryogenesis with liver degeneration, a phenotype similar to that of p65/RelA-deficient animals.[2–7] The exact embryonic stage at which liver degeneration and death occurs in these three mutants correlates with the extent of NF-κB inhibition obtained by the different knockouts. NEMO-deficient mice die during embryonic days E10–E11, IKK2-deficient mice at E12–E13, while p65/RelA knockouts die at E13–E15. As the gene encoding NEMO is located on the X-chromosome, the tissues of heterozygous NEMO knockout females contain both wild-type and NEMO-deficient cells in a mosaic pattern due to lyonisation. Heterozygous NEMO-deficient mice develop inflammatory skin lesions shortly after birth,[6,7] similarly to human patients with incontinentia pigmenti, a genetic disease caused by NEMO mutations.

The generation of mice lacking IKK1 yielded an unexpected phenotype not seen in any other NF-κB pathway component knockout. These mice die shortly after birth and display defective epidermal keratinocyte differentiation and skeletal abnormalities.[8–10] This phenotype was later shown to be independent of NF-κB activation and of inducible IKK1 kinase activity.[11] IKK1 was proposed to control epidermal keratinocyte differentiation via the regulation of an as yet unidentified factor (KDif), through a pathway different than NF-κB, the nature of which also remains elusive,[11] demonstrating that in the interpretation of knockout mouse phenotypes, NF-κB-independent activities of the respective proteins have to be taken into account. Complementation of the IKK1 knockout mice with transgenic expression of wild-type or catalytically inactive IKK1 in epidermal keratinocytes rescued the epidermal differentiation defect and also the skeletal phenotype, suggesting that IKK1 acts in the epidermis to control skeletal morphogenesis.[12]

The analysis of cells lacking individual IKK subunits led to the discovery of two distinct pathways regulating NF-κB activation. IKK2 and NEMO are essential for NF-κB activation via the 'canonical' pathway, which is induced by proinflammatory stimuli such as TNF, IL-1 and lipopolysaccharide (LPS), and leads to the phosphorylation and degradation of IκB proteins and the nuclear accumulation of NF-κB dimers containing predominantly p65/RelA, p50 and c-Rel (Figure 1). IKK1 seems to be dispensable for this process, but it regulates, in a NEMO- and IKK2-independent manner, NF-κB activation via the 'alternative' pathway, which is activated downstream of receptors controlling lymphoid organogenesis and lymphocyte development, such as the lymphotoxin-β receptor and the BAFF receptor,[13] and induces p100 processing, resulting in nuclear accumulation of mostly p52/RelB dimers[14] (Figure 1). Despite the apparent segregation of IKK2 and IKK1 function in regulating the canonical and alternative pathways, respectively, these two kinases also display a degree of functional redundancy, exemplified by the presence of residual proinflammatory signal-induced NF-κB activity in IKK2-deficient cells. This activity cannot be induced in cells lacking both IKK2 and IKK1, showing that IKK1 can partly compensate for the absence of IKK2 in activating NF-κB via the canonical pathway.[15]

Conditional knockout of IKK subunits

Conditional gene targeting combines features of classical gene knockouts and transgenesis to achieve spatially and temporally controlled gene manipulation. This technology is

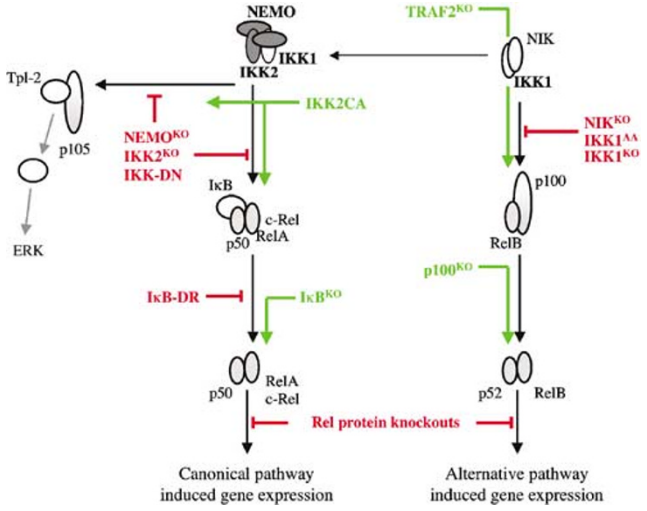

Figure 1 Approaches allowing inhibition or activation of the canonical and the alternative NF-κB activation pathways in transgenic mice. Activation of the canonical pathway results from overexpression of constitutively active IKK2-CA or from knock out of IκB proteins. Inhibition of the canonical pathway can be achieved by knockout of IKK2, NEMO or NF-κB/Rel proteins (p50, RelA, c-Rel), but also by overexpression of IκB-DR. The alternative pathway can be activated by knockout of TRAF2 or of the IκB-like domain of p100, and can be inhibited by knockout or mutation of IKK1 and NIK or by knockout of Rel proteins (p52, RelB). Green arrows indicate activation, and red lines indicate inhibition.

particularly useful when the classical knockout of a gene results in early lethality or in very complex phenotypes involving more than one tissue or cell type. Conditional gene targeting is based on the usage of the Cre/*loxP* recombination system to modify genomic sequences in a tissue-specific and/or inducible manner.[16] Using homologous recombination in mouse ES cells, sequences of any given gene can be flanked by *loxP* sites. Mice harbouring such *loxP*-flanked, or conditional, alleles are then crossed to transgenic mice expressing Cre under the control of tissue-specific promoters. In the offspring from such intercrosses, the conditional allele is inactivated through Cre-mediated recombination only in cells that express sufficient amounts of this enzyme.

The ubiquitous expression of IKK/NF-κB signalling components and the diverse functions they regulate, in combination with the embryonic or perinatal lethal phenotype of mutant mice lacking p65, NEMO, IKK2 or IKK1, makes the IKK/NF-κB system particularly suitable for analysis by conditional gene targeting. Mice carrying conditional *Ikk2* or *Nemo* alleles[6,17,18] have been used to study the function of these molecules in a variety of tissues such as B- and T-lymphocytes, myeloid cells, hepatocytes, skeletal muscle, epidermal keratinocytes, gut epithelial cells and neurons.[17–28] The conditional knockout of IKK subunits in B- and T-lymphocytes and in the liver will be discussed in more detail in later sections. Mice with deletion of IKK2 specifically in epidermal keratinocytes develop an inflammatory skin disease that requires TNF signalling, but occurs independently of T- and B-lymphocytes, suggesting that the primary role of IKK2-mediated NF-κB activation in the epidermis is to regulate immune homeostasis in the skin.[18] Recently, neuronal-specific ablation of IKK2 was reported to protect mice from ischemia-induced brain damage, showing that NF-κB activation in neurons mediates neuronal death during brain ischemia.[28]

Conditional ablation of IKK2 using *Lysozyme-Cre* transgenic knock-in mice has been used to investigate the function of NF-κB signalling in myeloid cells. These results, however, have to be viewed critically, considering that the *Lysozyme-Cre* line employed in these studies often does not lead to complete deletion of the conditional alleles and also may not target all different macrophage subpopulations with similar efficiency.[19,29–31] Inducible ablation of IKK2 using the *Mx-Cre* system has also been employed to study the role of IKK2 in myeloid cells. Upon induction, the *Mx-Cre* transgene mediates very efficient recombination in haematopoietic cells and in the liver, but additional deletion occurs in almost all mouse tissues with variable efficiencies.[32] Myeloid-specific ablation of IKK2, surprisingly, led to increased atherosclerosis in LDL-receptor-deficient mice.[31] Deletion of IKK2 in macrophages also demonstrated that IKK2-mediated NF-κB activity is important to protect macrophages from apoptosis induced by TLR4 signalling.[33]

Deletion of IKK2 specifically in the epithelium of the gut revealed the dual function of this kinase in protecting epithelial cells from death, but also in promoting systemic inflammation after ischemia.[26] Greten *et al*.[34] showed that deletion of IKK2 in the gut epithelium resulted in reduced tumour numbers in a mouse model of colon cancer, presumably due to increased apoptosis of epithelial cells during tumour promotion, and proposed that IKK2-mediated NF-κB activity is critical for the survival of cells during the early stages of carcinogenesis in this model. Deletion of IKK2 in myeloid cells did not affect the number of tumours arising in this model, but resulted in decreased tumour size, suggesting that IKK2-mediated NF-κB activity is required for the expression of proinflammatory cytokines by myeloid cells that act as growth factors for tumour cells.[34] Deletion of IKK2 in skeletal muscle failed to prevent obesity-induced insulin resistance, suggesting the IKK2 signalling in this tissue is not implicated in type 2 diabetes.[23]

NF-κB inhibition by transgenic overexpression of repressor or dominant-negative (DN) proteins

An approach that has been widely used to inhibit NF-κB activation in mice relies on the transgenic (over)expression of IκB proteins. Although overexpression of wild-type IκBs has been applied in certain cases, most of these studies employed transgenic expression of various degradation-resistant IκB mutants (collectively termed IκB-DR) that act in a DN manner to block NF-κB activation. Efficient blockade of NF-κB activation through the transgenic expression of IκB-DR proteins represents an important tool, since this protein should interfere preferentially with the activity of canonical NF-κB dimers (Figure 1). However, inhibition will most likely never be complete, due to the presence of endogenous IκB proteins.

Inhibition of NF-κB through transgenic expression of IκB-DR proteins has been applied in various tissues, including T- and B-lymphocytes, liver, epidermis, heart, skeletal muscle, astrocytes, neurons and pancreas[35–50] (Table 1). Individual transgenic mouse strains overexpressing IκB-DR proteins in a given tissue often show different phenotypes

of varying severity. In most cases, the levels of IκB-DR expression directly correlate with the extent of NF-κB inhibition achieved, therefore transgenic lines showing higher expression levels, for example, due to high-copy number transgene integration, result in more complete NF-κB inhibition and more severe phenotypes. In addition to the nature of the specific IκB-DR molecule employed, the usage of different promoter/enhancer constructs to drive IκB-DR expression in the same cell type usually results in diverse phenotypes due to variability in the specificity, timing and also levels of transgene expression. Variegation of transgene expression resulting in a nonhomogeneous level of inhibition within cells of a given tissue could also further complicate the interpretation of these studies. As an example for the value of using this approach to achieve NF-κB inhibition, the results obtained using IκB-DR expression in T- and B-lymphocytes and in the liver will be discussed in more detail in later sections, where they will be compared to the findings obtained using different methods such as the conditional knockouts of IKK subunits or overexpression of IKK-DN proteins.

An alternative approach to express IκB-DR in transgenic mice is to place the IκB-DR coding sequences under the control of an endogenous locus, in order to avoid problems related to the transgene integration site. Schmidt-Ulrich *et al*.[51] have used this approach to generate mice expressing IκB-DR under the control of the ubiquitously expressed β-catenin locus. The expression of IκB-DR in these mice can be activated upon Cre-mediated removal of a *loxP*-flanked transcriptional stop cassette, thus providing tissue-specific NF-κB inhibition based on the Cre transgenic line used. Upon Cre-mediated excision of the transcriptional stop cassette in all tissues, these mice display defects in the development of ectodermal appendages and also impaired immune responses.[51] A potential disadvantage of this particular approach is that NF-κB inhibition by expression of IκB-DR always occurs in the background of heterozygous β-catenin knockout, thus making it impossible to exclude contribution of a potentially combinatorial effect of NF-κB inhibition together with β-catenin heterozygosity to any observed phenotype.

Another means to achieve NF-κB inhibition is the overexpression of catalytically inactive IKK subunits, which can act in a DN manner to block IKK activation and IκB phosphorylation and degradation (IKK-DN). Such catalytically inactive IKKs have been generated by replacing the serines of the kinase activation loop with alanines, thus eliminating the inducible IKK activity. This approach has been used in B-[52] and T-lymphocytes,[53] as discussed later. Inducible expression of IKK2-DN in cortical neurons was recently shown to protect neurons from apoptosis during brain ischemia in a mouse model of stroke.[28]

Activation of NF-κB by transgenesis

Knockout of IκB proteins

The targeted inactivation of inhibitors of NF-κB has provided important information about the function of the different IκB proteins in regulating NF-κB signalling. The results obtained from the targeted inactivation of one or more IκB proteins in mice have been the subject of numerous comprehensive reviews (for example, see Li and Verma[1]) and will not be discussed here.

NF-κB activation through transgenic overexpression of constitutively active IKK2

NF-κB inhibition in transgenic and knockout mice has provided invaluable information on the role of this pathway in physiological and pathological processes. A complementary approach allowing the investigation of the function of NF-κB signalling *in vivo* is to use transgenic technology to activate, rather than inhibit, NF-κB in a given tissue. Analysing the effects of activation *versus* inhibition of NF-κB in the same cell type offers a powerful tool to elucidate NF-κB function. Transgenic expression of a constitutively active mutant IKK2 (IKK2CA), derived by replacing the two serine residues of the activation loop (Ser 177 and 181) by glutamic acid, has been employed as a means to activate NF-κB in a cell-autonomous and stimulation-independent manner (Figure 1). Using this approach to activate NF-κB in thymocytes, Voll *et al*.[54] showed that NF-κB activation could provide a survival signal downstream of the pre-TCR that is implicated in T-cell development. Forced activation of NF-κB via overexpression of IKK2CA in skeletal muscle uncovered the pathogenic function of NF-κB activity in muscle wasting.[48] Moreover, neuronal-specific NF-κB activation induced by expression of IKK2CA led to increased neuronal apoptosis in a mouse model of stroke, complementing a series of experiments employing NF-κB inhibition in the same tissue to show that IKK/NF-κB activation mediates ischemia-induced neuronal death.[28] Therefore, stimulus independent NF-κB activation by the expression of IKK2CA has proven extremely useful in unravelling the function of NF-κB in both physiological and pathological processes.

Transgenic analysis of NF-κB function using gene modifications by knock-ins

Current transgenic technology allows the manipulation of the mouse genome with nucleotide precision, thus making possible the generation of knock-in mice carrying specific modifications in selected genes. Cheng *et al*.[55] took advantage of the targeted knock-in technology to generate mice that express IκBβ under the control of the IκBα genomic locus. These authors reported that, surprisingly, these mice were apparently normal and did not display the phenotype of IκBα knockouts, and suggested that the different functions of IκBα and IκBβ in controlling NF-κB activity could be attributed almost entirely to their differential transcriptional regulation.[55]

A prime example for the usefulness of protein modification by directed mutagenesis of the mouse genome is the IKK1^{AA} mouse strain.[56] In these mice, the two serine residues at positions 176 and 180 within the kinase domain of IKK1 have been substituted for alanines, making the kinase refractory to activation, while preserving its basal kinase activity. This mutation allowed dissecting the inducible kinase activity of IKK1 from its structural, transcriptional and other potential functions. The IKK1^{AA} mice display normal epidermal differentiation demonstrating that IKK1 regulates this process independently of its inducible kinase activity.[56] The analysis

of the IKK1^{AA} mice revealed the critical role of inducible IKK1 kinase activity in controlling mammary epithelial proliferation in response to RANK signalling via cyclin D1,[56] and also for p100 processing via the alternative pathway.[14]

Monitoring NF-κB activity in transgenic mice

Transgenesis also offers the possibility to visualise NF-κB activation by placing a 'reporter protein' under the control of NF-κB-responsive *cis*-regulatory elements. This is particularly relevant for monitoring NF-κB activation *in vivo* and in small cell populations, where biochemical analysis can be tedious. The first transgenic mouse generated to monitor NF-κB transcriptional activity *in vivo* was reported by Lernbecher *et al*.,[57] who placed a β-globin gene under the control of a minimal promoter containing three NF-κB sites. Since then, various proteins have been used to report for NF-κB activity, among them β-galactosidase,[58,59] EGFP[60] and luciferase.[54,61–63]

A number of transgenic mouse strains have been generated expressing reporter proteins under the control of different NF-κB-responsive promoter constructs. It is likely that any single NF-κB-regulatory element will provide a read out of only a subset of endogenous NF-κB activities; therefore, it is not surprising to see differences between animals generated using distinct NF-κB *cis*-elements. For example, the analysis of β-galactosidase expression in the CNS during embryonic development and in adult mice in two different κB-LacZ transgenic lines, using either the p105 promoter/enhancer[58] or a combination of three tandem HIV-derived κB sites placed proximal to a minimal SV40 promoter,[59] revealed constitutive κB-driven expression in neurons of the adult CNS in both cases; however, strong widespread expression of β-galactosidase in the developing CNS was detected only in the latter study.

One of the main disadvantages of using such reporter proteins for monitoring NF-κB activation is the inability to follow NF-κB activation dynamics accurately. First, due to the time required for the expression and folding of the reporter proteins upon NF-κB activation, there is a delay before NF-κB activity can be visualised. Second, the reporter proteins are usually very stable and remain in cells after shutdown of NF-κB activity, thus failing to reproduce the rapid downregulation of NF-κB signalling that occurs in most cases. To overcome this problem, Gross *et al*.[64] generated mice expressing a fusion protein between luciferase and IκBα, which permits the direct visualisation of IKK activity. This strategy allows real-time monitoring of IKK activity by measuring IκBα degradation through the reduction of bioluminescence in cells and also *in vivo* in mice, thus providing a means to follow the temporal as well as the spatial regulation of IKK and NF-κB activity.

Examples of the Use of Transgenic Mouse Models to Study the Function of NF-κB Activation *In Vivo*

Mouse models to study the function of NF-κB in B-lymphocytes

Since NF-κB was first identified in B cells, it seems fitting that signalling leading to the activation of NF-κB/Rel transcription factors, and the subsequent biological effects, are may be best defined in the B-lineage. The state-of-the-art of NF-κB signalling in B-cell development and functions has been summarised in recent comprehensive reviews.[13,65] Here we will attempt to illustrate, in an exemplary manner, how the application of different genetic approaches in the mouse has contributed to the current understanding of the mechanisms and biology of NF-κB signalling in B cells.

Inhibition of canonical NF-κB activation in B cells

Most of our current knowledge about the role of NF-κB activation in B cells has been obtained through extensive analyses of single and compound NF-κB/Rel knockout mice. These studies have uncovered roles for NF-κB/Rel proteins in B-cell survival, proliferation and antibody production (reviewed in Siebenlist *et al*.[13] and Gerondakis and Strasser[66]).

Adoptive transfer of knockout bone marrow or foetal liver cells into irradiated recipient mice leads to the generation of chimeras carrying the genetic mutation restricted to cells of the haematopoietic system (subsequently called haematopoietic chimeras) and allows one to circumvent embryonic lethal phenotypes. No lymphocytes could be recovered from irradiated recipients of NEMO-deficient foetal liver cells,[67] consistent with the absence of lymphocytes lacking NEMO in chimeras generated through the injection of NEMO-deficient ES cells into wild-type blastocysts.[6] These findings demonstrated that NEMO-deficient haematopoietic stem cells, or early B-cell progenitors, require NEMO, or cannot compete with their NEMO-proficient counterparts. *In vitro* differentiation of NEMO-deficient ES cells indicated that immature B cells could develop independently of NEMO in a cell culture system.

Jimi *et al*.[68] attempted to address the role of canonical NF-κB signalling in early B cells, by expressing IκBα-DR under the control of mb-1 promoter and Eμ-enhancer sequences. Using this strategy, they achieved a mild reduction in pro-B-cell numbers, but IκBα-DR levels never reached levels equivalent to endogenous IκBα. Transgenic expression of IκBα-DR under the control of an immunoglobulin (Ig) heavy-chain promoter/intronic enhancer cassette by Bendall *et al*.[69] led to a reduction of IgD$^+$ B cells in spleen and bone marrow. IκBα-DR expression inhibited αIgM-, but not LPS-induced thymidine incorporation by cultured B cells in a dose-dependent manner. Corresponding to its impact on proliferation, IκBα-DR inhibited NF-κB DNA binding in response to αIgM, but not to LPS.

Interference with NF-κB activation through B-cell-restricted expression of IKK2-DN yielded completely different results.[52] The IKK2-DN protein efficiently inhibited NF-κB activation in response to αIgM or LPS, apparently better than the IκBα-DR did. However, IKK2-DN expression had no effect on B-cell development and inhibited thymidine incorporation in response to LPS, but had only small effects on the responses to stimulation with αIgM or αCD40. Mice expressing IKK2-DN in B cells produced less antigen-specific class-switched Igs than controls after immunisations with type 2 T-independent and T-dependent antigens, respectively. The underlying mechanisms of this defect remain unclear, since the subtle differences in class switching that were observed *in vitro* between

IKK2-DN and control B cells were attributed to the reduced proliferation of the former.[52]

Two groups have independently addressed the role of the canonical NF-κB pathway in B cells through inactivation of *Ikk2*^{FL} [17,27] and *Nemo*^{FL} [27] conditional alleles by Cre expressed from the endogenous CD19 promoter.[17,27] Four individual alleles were used in these studies, here collectively referred to as *IKK*^{FL} (standing for *Ikk2*$^{FL/FL}$,[17] *Ikk2*$^{FL/D}$, *Ikk2-ΔK*$^{FL/D}$ and *Nemo*$^{FL/Y}$ [27]). *CD19Cre* mice facilitate excision of *loxP*-flanked DNA sequences exclusively in B cells with high efficiency. In case of the *CD19Cre/IKK*^{FL} mice, early B-cell development occurs normally, but all splenic B-cell populations are reduced in size, especially the mature follicular and marginal zone B-cell subsets. Southern blot analyses showed considerable proportions of B cells retaining *loxP*-flanked, IKK2 (or NEMO)-expressing alleles. As the *CD19Cre* transgene mediated very efficient recombination of control heterozygous conditional alleles, the incomplete deletion observed with the IKK alleles was a clear indication that IKK-deficient B cells were counter-selected. Taking into account that, after Cre-mediated excision of a given conditional allele, cells will become true 'knockout' cells only after they have lost the gene products of this allele, namely steady-state mRNA and protein, through turnover, the detection of B cells with recombined IKK alleles does not automatically indicate that these cells are also deficient for IKK signalling. The finding that B-cell turn over was accelerated in *CD19Cre/IKK*^{FL} mice, together with the complete loss of IKK2-deficient splenic B cells upon anti-IL7R antibody-mediated blockade of B-cell influx from the bone marrow, demonstrated that canonical NF-κB activation is essential for the maintenance of mature B cells.[27]

Induction of canonical NF-κB activity in B cells

Haematopoietic chimeras generated with *IκBα*$^{-/-}$ foetal liver cells[70] showed normal B-cell development, but displayed increased basal serum immunoglobulin levels and increased B-cell proliferation upon *in vitro* stimulation with αCD40, αIgM and LPS. However, *IκBα*$^{-/-}$ haematopoietic chimeras showed impaired immune responses to immunisation with T-dependent and T-independent antigens. *IκBα/ε*$^{-/-}$ foetal liver cells could not give rise to lymphocytes in *Rag2/γc*$^{-/-}$ recipients, even when wild-type bone marrow was cotransferred, suggesting that excessive NF-κB activity is incompatible with early lymphocyte development.[71]

Ishikawa *et al*.[72] generated mice that lack the IκB-like C-terminal part of p105, and therefore constitutively express p50. These mice display a complex inflammatory phenotype with increased mortality. Both basal and antigen-specific serum Ig levels are elevated, while *p105*$^{-/-}$ B cells show increased proliferation in response to antigen receptor cross-linking and LPS.[72] However, it remains unclear whether the underlying cause is a direct effect of the mutation on B cells, or an indirect effect of the multi-organ inflammation. Activation of the IKK complex mostly leads to complete proteolysis of p105, thereby liberating Tpl-2, which in turn activates the ERK signalling cascade[65] (Figure 1). Since p105 is also required to stabilise Tpl-2,[65] the phenotype of *p105*$^{-/-}$ mice could be a result of constitutive activation of p50 homodimers in combination with defective ERK activation.

Mouse models for canonical IKK signalling in B cells: lessons and challenges

The absence of any alteration in early B-cell development in the bone marrow of *CD19Cre/IKK*^{FL} mice,[17,27] together with the minor effects seen by IκBα-DR expression,[68] and the *in vitro* findings of Kim *et al*.,[67] collectively suggest that canonical NF-κB activation is not required for this process. Results obtained from chimeras generated with NEMO-deficient stem cells[6,67] and from retroviral overexpression of IκBα-DR in bone marrow-derived stem cells,[68] point to a critical role for canonical NF-κB in lymphopoiesis prior to the commitment to the B-cell lineage. Therefore, improved mouse models are required to fully clarify the *in vivo* role of canonical NF-κB activity in bone marrow B-cell development, such as a mouse strain facilitating complete Cre-mediated protein ablation in early B-cell precursors.

Attempts to elucidate the function of canonical NF-κB activity in mature B cells, through transgenic expression of IκBα-DR[69] or IKK2-DN,[52] yielded conflicting results. The effectiveness of IκBα-DR and IKK2-DN to inhibit NF-κB activation induced by *in vitro* stimulation did not correlate very well with the impact on B-cell development and/or maintenance, demonstrating that measuring inhibition of inducible NF-κB activity *in vitro* is not necessarily a good indicator for the impact on NF-κB activation in a more physiological context. Although these studies described the involvement of canonical NF-κB in different aspects of B-cell physiology, as would be expected from the defects observed in B cells lacking one or more NF-κB/Rel subunits, the contribution of canonical NF-κB signalling was most likely underestimated due to the presence, and residual function, of endogenous IκBα and IKK2, respectively.

Conditional gene targeting revealed that B-cell maintenance requires intact canonical NF-κB activation,[27] but the experimental system did not permit definite assignment of other functions, since B cells purified from the spleens of *CD19Cre/IKK*^{FL} mice contain significant proportions of IKK-proficient cells. Further roles of IKK-mediated NF-κB activation in B cells could be defined if the survival defect inferred by defective IKK signalling can be overcome, for example, by enforced expression of NF-κB-dependent anti-apoptotic proteins.

Aberrant constitutive activation of the IKK complex and canonical NF-κB activity is found in activated B-cell-like diffuse large B-cell lymphoma, MALT lymphomas and in Hodgkin's and Reed–Sternberg cells.[73] To define the role of canonical NF-κB activity in B cells, especially in the context of B-cell transformation, additional mouse models, such as expression of a constitutively active IKK2, or conditional ablation of IκBα on an IκBε-deficient background, are required.

Alternative NF-κB signalling depends on NIK and IKK1

Chimeras containing a haematopoietic compartment expressing a mutant form of NIK (*aly/aly*),[74] or lacking IKK1,[14,75] show similar phenotypes: Severe reduction in peripheral B-cell numbers and defects in B-cell function. IKK1^{AA} mice essentially recapitulate the phenotypes seen in IKK1-knockout haematopoietic chimeras, confirming that the kinase activity of IKK1 is required for proper B-cell maturation and function.[14] NIK and IKK1 are required for the establishment of

splenic microarchitecture, terminal differentiation and/or survival of mature B cells, surface expression of CD21 and germinal centre formation and antigen-specific IgG1 production in response to immunisations. However, B cells purified from *aly/aly* mice produced elevated amounts of IgG2b and IgG3 when stimulated with LPS, and IgG1 upon αCD40/IL-4 treatment, suggesting that NIK has no direct role in class switching. Similarly to *aly/aly* splenocytes,[74] *Ikk1*$^{-/-}$ B cells do not contain p52, but unlike the former show elevated levels of p100 and normal levels of c-Rel.[14] c-Rel protein levels are strongly reduced in the absence of NIK function.[74] Early B-cell development in the bone marrow is independent of IKK1, whereas *aly/aly* haematopoietic chimeras contain a reduced fraction of IgM^{+} immature B cells in the bone marrow.

Turning on alternative NF-κB activity

The deletion of the C-terminal ankyrin repeats of *p100* leads to production of p52 in the absence of the inhibitory domain of p100,[76] which mimics, to some extent, activation of alternative NF-κB signalling. These *p100*$^{-/-}$ mice, however, develop gastric hyperplasia, become severely sick and most die within the first month after birth. Therefore, it remains unclear to what extent the observed haematopoietic phenotypes are secondary to the gastric condition.[76] The generation of haematopoietic chimeras with *p100*$^{-/-}$ stem cells should be very informative, but has not yet been reported.

The power of selection within the haematopoietic system is exemplified in *MxCre/Traf2*$^{FL/FL}$ mice.[77] Low-level spontaneous Cre expression from the *Mx1* promoter,[78] in the absence of inducer, results in 'leaky deletion' of target alleles typically in a very small fraction of cells. In *MxCre/Traf2*$^{FL/FL}$ bone marrow, a small percentage of recombined *traf2*Δ alleles can be detected in immature B cells, whereas, in striking contrast, all splenic B cells are TRAF2 deficient. Since very little Cre-mediated recombination occurs in mature *MxCre/Traf2*$^{FL/WT}$ B cells, the loss of TRAF2 must provide splenic B cells with a tremendous advantage over their TRAF-2-expressing counterparts, leading to their preferential selection. The advantage induced by lack of TRAF2 turns out to be a BAFF-independent maximal activation of the alternative NF-κB pathway (Figure 1). Further analysis showed that TRAF2 inhibits activation of the alternative pathway in resting B cells, but is required for CD40-induced canonical NF-κB activity. Lack of TRAF2 also leads to increased levels of TRAF3, c-Rel and Bcl-xL. As a consequence of these biochemical changes, the spleens of *MxCre/Traf2*$^{FL/FL}$ mice contain twice the amount of follicular B cells compared to control mice, whereas marginal zone B-cell numbers are increased by a factor of five and some TRAF2-deficient B cells with a surface phenotype reminiscent of marginal zone B cells are found in the lymph nodes. *Traf2*$^{-/-}$ B cells are larger and survive much better under cell-culture conditions than control B cells, indicating that enhanced survival could contribute to the observed B-cell hyperplasia.

Mouse models to study alternative NF-κB activation in B cells

The B-cell deficiencies observed in *aly/aly* and *Ikk1*$^{-/-}$ haematopoietic chimeras and in IKK1^{AA} mice were attributed to incapacitation of the alternative pathway of NF-κB activation.[14] However, to what extent the observed B-cell phenotypes are due to B-cell-autonomous mechanisms, or pertain to functional defects in other haematopoietic cell types, remains unresolved. The analysis of TRAF-2-deficient B cells yielded insights into the consequences of constitutive activation of alternative NF-κB, but it is likely that TRAF2 deficiency affects additional signalling pathways. New mouse models are required to pinpoint the effects of alternative NF-κB signalling, such as mice permitting the conditional activation and inactivation of IKK1 or NIK, or the conditional truncation of p100, since such mutant forms of p100 were found in some B- and T-cell lymphomas.[79]

Mouse models to study the function of NF-κB in T-lymphocytes

The analysis of knockout mice lacking single or combinations of two NF-κB/Rel proteins revealed important roles for NF-κB/Rel subunits in T-cell physiology, reviewed in Siebenlist *et al*.[13]

The function of canonical NF-κB activation in cells of the T-lineage was studied extensively in transgenic mice expressing wild-type or mutant IκB proteins: wild-type IκBα,[37] truncated IκBα,[35] mIκBα^{S32A,S36A} [36,80,81], mIκBβ^{S19A,S23A} [38] and a super-repressor (IκBα-SR), in which all known amino acids associated with induced protein degradation, and the serines of the PEST domain, had been mutated.[54] The respective proteins were placed under the transcriptional control of various expression constructs: Ferreira *et al*.[36] used the proximal lck promoter in the context of a human growth hormone minigene. This construct, with the addition of the locus control region (LCR) of the human CD2 gene was employed by three other groups.[35,38,80] Hettmann *et al*.[81] used a human CD2 promoter and LCR construct, while Esslinger *et al*.[37] combined a β-globin promoter with the human CD2 LCR. All of these transgenic constructs mediated expression mostly in thymocytes and T cells, although in some cases transgene expression was also detected in B cells[37,80] or NK cells.[82] IκB-DR-expression led to a reduction in peripheral T-cell numbers of varying degrees, from very mild[81] to dramatic.[37] One common feature in these transgenic mice is that in the thymus only CD8 single-positive cells are reduced, if at all, and that peripheral CD8 T cells are more diminished than CD4 T cells. The most severe inhibition of T-cell generation was achieved in a transgenic mouse line carrying around 80 copies of a wild-type IκBα expression construct,[37] and this defect could not be rescued through enforced expression of Bcl-2.[83] The functional consequences of IκB-DR expression differed considerably between the individual mouse strains, but all transgenic T cells displayed defects in proliferation and cytokine production. However, the severity of the defects caused by inhibition of NF-κB in these studies may have been underestimated, as more detailed examination of transgenic T-cell proliferation by Tato *et al*.[82] revealed that proliferating T cells had low or no expression of the transgene, which was readily detected in the nondividing cells. Therefore, variegation of transgene expression might complicate the interpretation of the results obtained from these studies.

Transgenic expression of DN versions of IKK1 or IKK2 under control of a human CD2 minigene did not interfere with T-cell development, even when the two transgenes were coexpressed, raising doubts whether IKK signalling is essential for T-cell development.[53] Indeed, thymocytes developed normally in IKK2/TNFRI-double-deficient mice[84] and adoptive transfer of IKK1-deficient foetal liver cells showed that peripheral T cells develop in the absence of IKK1.[75]

The role of the IKK complex in T-lymphocytes was further analysed by conditional ablation of IKK2 and NEMO using the *CD4Cre* transgenic mice.[24] Mice with T-cell-restricted NEMO ablation showed an almost complete lack of peripheral T cells, and the very few T cells present in the spleen and lymph nodes were shown to have escaped Cre-mediated recombination.[24] In contrast, naïve peripheral T cells developed in the absence of IKK2; however, distinct populations of memory-type, NK-like T (NKT) and regulatory T cells were absent in these mice.[24,85] Transgenic expression of IκB-DR also interferes with NKT cell development,[86] confirming that IKK2-mediated canonical NF-κB activity is essential for this T-cell subset. Conditional deletion of exon 6 of the *Ikk2* gene in T cells led to the production of a kinase-dead truncated IKK2ΔK, which, although expressed in reduced levels, caused a lack of peripheral T cells similar to the T-cell-restricted knockout of NEMO. These results showed that NEMO-dependent IKK signalling is essential for the development of mature T cells, and that expression of IKK2 or IKK1 alone is sufficient to mediate T-cell development. Expression of a kinase-dead IKK2, even at low levels, in the absence of endogenous IKK2, is sufficient to block T-cell development, which further supports a functional redundancy of the two IKKs in this function. T-cell-restricted knockout of both IKK1 and IKK2 could clarify the functional redundancies between these two kinases in T-lymphocytes.

Transgenic analysis of the role of NF-κB signalling in the liver

Several recent studies have suggested that cytokines and cytokine-activated inflammatory signalling pathways might play an important role in mediating liver injury. The liver is a complex organ composed of different cell types including hepatocytes, bile duct epithelial cells, endothelial cells and Kupffer cells, therefore *in vitro* analyses of cell lines cannot adequately address the function of NF-κB in liver physiology and disease. The use of transgenic and knockout mice in numerous studies has allowed a more physiologic insight into the role of the NF-κB pathway in the liver, a few of which are summarised here.

NF-κB in cytokine-induced hepatic failure

Death receptor ligands such as TNF or Fas ligand are involved in the induction of apoptosis and liver destruction, ultimately leading to hepatic failure. A single-dose injection of a Fas-activating antibody can lead to cell death in parenchymal and nonparenchymal liver cells.[87] In contrast to Fas-mediated apoptosis, hepatocytes are resistant to apoptosis induced by low doses of TNF or LPS, a potent inducer for endogenous TNF in the liver.[88] However, when cells or animals are treated with inhibitors of transcription such as cycloheximide, actinomycin D or D-galactosamine, even very low doses of TNF or LPS result in massive hepatocyte apoptosis and lethal liver failure respectively.[89,90] These studies demonstrated that the activation of a transcriptional programme in the liver is essential to provide prosurvival signals to counter balance the death-inducing capacity of TNF.

Evidence that NF-κB is critical for the protection of hepatocytes from TNF-induced apoptosis came from the finding that mice lacking p65/RelA die during embryogenesis by TNF-induced hepatocyte apoptosis.[91] This phenotype was later also observed in mice lacking IKK2[2–4] or NEMO.[5–7] Therefore, during embryogenesis p65/RelA and the IKK2 and NEMO subunits of the IKK complex are critical for NF-κB activation and protection of embryonic liver cells from TNF-induced apoptosis.

The finding that NF-κB activity is important for protection of cells from TNF-induced apoptosis triggered efforts to study the function of NF-κB in hepatic failure in adult mice. Several groups used transgenic mice expressing IκBα-DR to inhibit specifically the NF-κB pathway in the liver. Lavon *et al*.[40] generated a mouse strain expressing an IκBα-DR transgene in mouse hepatocytes in a tetracycline-responsive manner. When IκBα-DR was expressed, these mice were vulnerable to liver damage caused by injection of ConA, which mediates hepatocyte apoptosis via the induction of TNF expression by both T-lymphocytes and myeloid cells,[92] showing that NF-κB is important for the protection of hepatocytes from TNF-induced death *in vivo*. Chaisson *et al*.[43] followed a similar approach by generating a mouse strain, in which IκBα-DR expression could be induced in hepatocytes by injection of mifepristone. They demonstrated that NF-κB activation upon TNF stimulation could be blocked *in vivo* in 45% of hepatocytes, leading to apoptosis 4 h after TNF stimulation. Collectively, these studies showed that NF-κB inhibition by overexpression of IκBα-DR sensitises hepatocytes to TNF-induced apoptosis.

Recently, the role of IKK signalling in cytokine-induced liver failure was addressed using hepatocyte-specific ablation of IKK2 or NEMO. Maeda *et al*.[25] found that mice lacking IKK2 in hepatocytes were not sensitive to LPS-induced liver toxicity, but were susceptible to ConA-induced liver damage. Based on this observation, they proceeded to show that in the absence of IKK2, ConA causes liver toxicity through sustained activation of JNK, induced by binding of membrane-bound TNF to both TNFR-I and -II. Using a different conditional *Ikk2* allele, Luedde *et al*.[20] have recently reported that mice lacking IKK2 in the liver are not sensitive to liver damage caused by injection of recombinant TNF. In contrast, injection of similar amounts of TNF in mice lacking NEMO in the liver caused dramatic hepatocyte apoptosis and liver toxicity.[20] Consistent with these findings, NF-κB activation was completely inhibited in NEMO-deficient hepatocytes, while IKK2 knockout hepatocytes displayed significant, albeit reduced, NF-κB activity induced by TNF stimulation *in vivo* or *in vitro*. Based on these results, Luedde *et al*.[24] hypothesised that in hepatocytes, as seen in T cells, IKK1 can partly compensate for the absence of IKK2 in inducing NF-κB activation and protection from death upon TNF stimulation. The generation of mice with

hepatocyte-restricted ablation of both IKK1 and IKK2 will be useful to address the redundancies in the functions of these kinases in the liver.

NF-κB in liver regeneration

A well-established model to study liver regeneration is the partial (two-thirds) hepatectomy (PH) in rodents. The involvement of TNF and NF-κB in this process was suggested by the fact that liver regeneration is defective in TNFR-1 knockout mice, which do not show hepatic NF-κB activation after PH.[93] Indeed, NF-κB was initially identified in the liver as a factor that is rapidly activated within 30 min after PH. Further efforts were aimed at a more specific inhibition of the NF-κB pathway in this model. Inhibition of NF-κB by adenoviral gene transfer of IκBα-DR into mouse livers led to a defect in cellular proliferation and increased apoptosis after PH.[94] Given these clear indications for a proproliferative function of NF-κB in the liver, it was rather surprising that hepatocyte-specific transgenic expression of IκBα-DR did not impair hepatocyte proliferation or lead to increased apoptosis following PH.[43] Although it is possible that the adenoviral infection itself might contribute to hepatocyte apoptosis, another explanation for this apparent discrepancy appears more likely: Adenoviral vectors infect both parenchymal and nonparenchymal liver cells,[95,96] whereas in the transgenic mouse model, IκBα-DR was expressed specifically in hepatocytes.[43] Numerous previous studies have highlighted the important role that nonparenchymal liver cells, such as Kupffer cells, a macrophage-like cell type in the liver, might play in regulating the proliferative response of hepatocytes after PH.[97] Therefore, NF-κB could have different roles in the various cell types of the liver in the context of regeneration after PH.

This notion was recently confirmed through application of the conditional knockout technology. A study by Maeda *et al*.[25] showed that loss of IKK2 in hepatocytes did not cause defects in liver regeneration after PH compared to control animals. In striking contrast, ablation of IKK2 in hepatocytes, haematopoietic cells and many other cell types including Kupffer cells with the *Mx-Cre* transgene strongly impaired liver regeneration.[19] This study suggests that IKK2-mediated NF-κB activation is involved in the production of cytokines like TNF and IL-6 by nonparenchymal liver cells, which in turn promote proliferation of hepatocytes, but has no cell-intrinsic role in hepatocyte proliferation.

NF-κB in models of liver cancer and diabetes

Recent studies using transgenic and conditional knockout mice for members of the TNF/NF-κB axis have highlighted the essential function of this pathway in the initiation and promotion of carcinogenesis in the liver. Pikarsky *et al*. studied the function of TNF/NF-κB signalling in inflammation-induced liver tumours using the MDR-2 knockout mice, which develop chronic cholangitis and subsequently hepatocellular carcinomas.[98] Blockade of NF-κB activation through overexpression of IκBα-DR in *Mdr-2*$^{-/-}$ mice during early stages of carcinogenesis had no effect on tumour formation. However, NF-κB inhibition during later stages of tumorigenesis markedly decreased the number and size of tumours compared to control mice.[98] In a parallel investigation, Maeda *et al*.[19] examined the role of IKK2-signalling in hepatocytes compared to nonparenchymal liver cells like Kupffer cells in a model of chemically induced liver carcinogenesis. They could demonstrate that after the application of the carcinogenic drug diethylnitrosamine to young mice, hepatocytes undergo apoptosis leading to compensatory proliferation of surviving cells. This process was enhanced in mice with hepatocyte-restricted IKK2 knockout, thus leading to more and larger liver tumours compared to wild-type mice. In contrast, when IKK2 was ablated from both hepatocytes and haematopoietic cells (and in most other cell types to varying extents), using the *Mx-Cre* transgene, mice developed less and smaller tumours, suggesting that systemic inhibition of IKK2 activity protects from carcinogenesis in this model by reducing the expression of proinflammatory mediators such as IL-6 from Kupffer cells.[19]

Inflammation is implicated in the pathogenesis of insulin resistance and type 1 diabetes. Cai *et al*.[49] demonstrated that constitutive activation of NF-κB in the liver by transgenic, hepatocyte-specific overexpression of IKK2CA led to an increased local and systemic insulin resistance in mice that otherwise were fed with a normal diet. Expression of IκB-DR in the liver could protect mice from insulin resistance induced by IKK2CA expression, or by high fat diet.[49] Arkan *et al*.[21] used conditional knockout of IKK2 to show that inhibition of NF-κB activation in hepatocytes prevents the development of insulin resistance in the liver, but not in muscle or fat. In the same study, myeloid-cell specific knockout of IKK2 protected mice from systemic insulin resistance.[21] These studies suggest that NF-κB induces the expression of inflammatory cytokines in myeloid cells, which then act on hepatocytes, apparently via NF-κB-dependent mechanisms, to induce insulin resistance, and provide a direct link between NF-κB-controlled inflammation and type 2 diabetes.

Concluding Remarks

In this review, we have attempted to illustrate how manipulation of the mouse genome has been used to study the NF-κB signalling pathway. A large number of transgenic mouse strains have been generated during the recent years that permit the dissection of various aspects of NF-κB function in a cell-type-specific manner (see Table 1). Many of these studies focused on investigating the role of NF-κB in disease pathogenesis. The selective inhibition or activation of the different modes of NF-κB signalling in these mouse models has yielded invaluable information about the role of this pathway in disease initiation and progression. As the NF-κB pathway is implicated in various pathologies, particularly inflammatory and autoimmune diseases and cancer, genetically modified mouse models will continue to play a pivotal role in the evaluation of the role of NF-κB in disease pathogenesis. The generation of new mouse models allowing the more accurate and ideally reversible manipulation of NF-κB signalling will be particularly important, in order to evaluate the potential therapeutic value of interfering with NF-κB activity in established disease.

Undoubtedly, the list of available mouse models will expand in the future as our growing understanding of the events

leading to the activation, but also to the negative regulation of NF-κB-dependent transcriptional activities, will prompt the design of novel sophisticated mouse strains. The generation of mice carrying targeted point mutations in NF-κB proteins themselves, cofactors or upstream regulators will provide essential information on the role of regulatory modifications, such as phosphorylation or ubiquitination, in the control of NF-κB activity. Furthermore, new mouse lines will be needed to study the mechanisms by which specificity of NF-κB-dependent gene regulation is achieved. In particular, the role of individual κB sites in a given target gene can be studied by generating mice carrying targeted modifications of NF-κB sites in endogenous transcriptional units in their original chromosomal context. Such mice will be invaluable in elucidating the mechanisms by which the specific sequence, the positioning and also the total number of NF-κB sites present in a given gene can determine its specific mode of regulation. In conclusion, genetically modified mouse mutants will continue to play a pivotal role in our efforts to understand the complex biological functions of this fascinating signalling cascade.

References

1. Li Q, Verma IM (2002) NF-kappaB regulation in the immune system. Nat. Rev. Immunol. 2: 725–734.
2. Li Q, Van Antwerp D, Mercurio F, Lee KF, Verma IM (1999) Severe liver degeneration in mice lacking the IkappaB kinase 2 gene. Science 284: 321–325.
3. Li ZW, Chu W, Hu Y, Delhase M, Deerinck T, Ellisman M, Johnson R, Karin M (1999) The IKKbeta subunit of IkappaB kinase (IKK) is essential for nuclear factor kappaB activation and prevention of apoptosis. J. Exp. Med. 189: 1839–1845.
4. Tanaka M, Fuentes ME, Yamaguchi K, Durnin MH, Dalrymple SA, Hardy KL, Goeddel DV (1999) Embryonic lethality, liver degeneration, and impaired NF-kappa B activation in IKK-beta-deficient mice. Immunity 10: 421–429.
5. Rudolph D, Yeh WC, Wakeham A, Rudolph B, Nallainathan D, Potter J, Elia AJ, Mak TW (2000) Severe liver degeneration and lack of NF-kappaB activation in NEMO/IKKgamma-deficient mice. Genes Dev. 14: 854–862.
6. Schmidt-Supprian M, Bloch W, Courtois G, Addicks K, Israel A, Rajewsky K, Pasparakis M (2000) NEMO/IKK gamma-deficient mice model incontinentia pigmenti. Mol. Cell 5: 981–992.
7. Makris C, Godfrey VL, Krahn-Senftleben G, Takahashi T, Roberts JL, Schwarz T, Feng L, Johnson RS, Karin M (2000) Female mice heterozygous for IKK gamma/NEMO deficiencies develop a dermatopathy similar to the human X-linked disorder incontinentia pigmenti. Mol. Cell 5: 969–979.
8. Takeda K, Takeuchi O, Tsujimura T, Itami S, Adachi O, Kawai T, Sanjo H, Yoshikawa K, Terada N, Akira S (1999) Limb and skin abnormalities in mice lacking IKKalpha. Science 284: 313–316.
9. Li Q, Lu Q, Hwang JY, Buscher D, Lee KF, Izpisua-Belmonte JC, Verma IM (1999) IKK1-deficient mice exhibit abnormal development of skin and skeleton. Genes Dev. 13: 1322–1328.
10. Hu Y, Baud V, Delhase M, Zhang P, Deerinck T, Ellisman M, Johnson R, Karin M (1999) Abnormal morphogenesis but intact IKK activation in mice lacking the IKKalpha subunit of IkappaB kinase. Science 284: 316–320.
11. Hu Y, Baud V, Oga T, Kim KI, Yoshida K, Karin M (2001) IKKalpha controls formation of the epidermis independently of NF-kappaB. Nature 410: 710–714.
12. Sil AK, Maeda S, Sano Y, Roop DR, Karin M (2004) IkappaB kinase-alpha acts in the epidermis to control skeletal and craniofacial morphogenesis. Nature 428: 660–664.
13. Siebenlist U, Brown K, Claudio E (2005) Control of lymphocyte development by nuclear factor-kappaB. Nat. Rev. Immunol. 5: 435–445.
14. Senftleben U, Cao Y, Xiao G, Greten FR, Krahn G, Bonizzi G, Chen Y, Hu Y, Fong A, Sun SC, Karin M (2001) Activation by IKKalpha of a second, evolutionary conserved, NF-kappa B signaling pathway. Science 293: 1495–1499.
15. Li Q, Estepa G, Memet S, Israel A, Verma IM (2000) Complete lack of NF-kappaB activity in IKK1 and IKK2 double-deficient mice: additional defect in neurulation. Genes Dev. 14: 1729–1733.
16. Rajewsky K, Gu H, Kuhn R, Betz UA, Muller W, Roes J, Schwenk F (1996) Conditional gene targeting. J. Clin. Invest. 98: 600–603.
17. Li ZW, Omori SA, Labuda T, Karin M, Rickert RC (2003) IKK beta is required for peripheral B cell survival and proliferation. J. Immunol. 170: 4630–4637.
18. Pasparakis M, Courtois G, Hafner M, Schmidt-Supprian M, Nenci A, Toksoy A, Krampert M, Goebeler M, Gillitzer R, Israel A, Krieg T, Rajewsky K, Haase I (2002) TNF-mediated inflammatory skin disease in mice with epidermis-specific deletion of IKK2. Nature 417: 861–866.
19. Maeda S, Kamata H, Luo JL, Leffert H, Karin M (2005) IKKbeta couples hepatocyte death to cytokine-driven compensatory proliferation that promotes chemical hepatocarcinogenesis. Cell 121: 977–990.
20. Luedde T, Assmus U, Wustefeld T, Meyer ZV, Roskams T, Schmidt-Supprian M, Rajewsky K, Brenner DA, Manns MP, Pasparakis M, Trautwein C (2005) Deletion of IKK2 in hepatocytes does not sensitize these cells to TNF-induced apoptosis but protects from ischemia/reperfusion injury. J. Clin. Invest. 115: 849–859.
21. Arkan MC, Hevener AL, Greten FR, Maeda S, Li ZW, Long JM, Wynshaw-Boris A, Poli G, Olefsky J, Karin M (2005) IKK-beta links inflammation to obesity-induced insulin resistance. Nat. Med. 11: 191–198.
22. Schmidt-Supprian M, Tian J, Ji H, Terhorst C, Bhan AK, Grant EP, Pasparakis M, Casola S, Coyle AJ, Rajewsky K (2004) I kappa B kinase 2 deficiency in T cells leads to defects in priming, B cell help, germinal center reactions, and homeostatic expansion. J. Immunol. 173: 1612–1619.
23. Rohl M, Pasparakis M, Baudler S, Baumgartl J, Gautam D, Huth M, De Lorenzi R, Krone W, Rajewsky K, Bruning JC (2004) Conditional disruption of IkappaB kinase 2 fails to prevent obesity-induced insulin resistance. J. Clin. Invest. 113: 474–481.
24. Schmidt-Supprian M, Courtois G, Tian J, Coyle AJ, Israel A, Rajewsky K, Pasparakis M (2003) Mature T cells depend on signaling through the IKK complex. Immunity 19: 377–389.
25. Maeda S, Chang L, Li ZW, Luo JL, Leffert H, Karin M (2003) IKKbeta is required for prevention of apoptosis mediated by cell-bound but not by circulating TNFalpha. Immunity 19: 725–737.
26. Chen LW, Egan L, Li ZW, Greten FR, Kagnoff MF, Karin M (2003) The two faces of IKK and NF-kappaB inhibition: prevention of systemic inflammation but increased local injury following intestinal ischemia-reperfusion. Nat. Med. 9: 575–581.
27. Pasparakis M, Schmidt-Supprian M, Rajewsky K (2002) IkappaB kinase signaling is essential for maintenance of mature B cells. J. Exp. Med. 196: 743–752.
28. Herrmann O, Baumann B, de Lorenzi R, Muhammad S, Zhang W, Kleesiek J, Malfertheiner M, Kohrmann M, Potrovita I, Maegele I, Beyer C, Burke JR, Hasan MT, Bujard H, Wirth T, Pasparakis M, Schwaninger M (2005) IKK mediates ischemia-induced neuronal death. Nat. Med. 11: 1322–1329.
29. Clausen BE, Burkhardt C, Reith W, Renkawitz R, Forster I (1999) Conditional gene targeting in macrophages and granulocytes using LysMcre mice. Transgenic Res. 8: 265–277.
30. Ye M, Iwasaki H, Laiosa CV, Stadtfeld M, Xie H, Heck S, Clausen B, Akashi K, Graf T (2003) Hematopoietic stem cells expressing the myeloid lysozyme gene retain long-term, multilineage repopulation potential. Immunity 19: 689–699.
31. Kanters E, Pasparakis M, Gijbels MJ, Vergouwe MN, Partouns-Hendriks I, Fijneman RJ, Clausen BE, Forster I, Kockx MM, Rajewsky K, Kraal G, Hofker MH, de Winther MP (2003) Inhibition of NF-kappaB activation in macrophages increases atherosclerosis in LDL receptor-deficient mice. J. Clin. Invest. 112: 1176–1185.
32. Kuhn R, Torres RM (2002) Cre/loxP recombination system and gene targeting. Methods Mol. Biol. 180: 175–204.
33. Park JM, Greten FR, Wong A, Westrick RJ, Arthur JS, Otsu K, Hoffmann A, Montminy M, Karin M (2005) Signaling pathways and genes that inhibit pathogen-induced macrophage apoptosis – CREB and NF-kappaB as key regulators. Immunity 23: 319–329.
34. Greten FR, Eckmann L, Greten TF, Park JM, Li ZW, Egan LJ, Kagnoff MF, Karin M (2004) IKKbeta links inflammation and tumorigenesis in a mouse model of colitis-associated cancer. Cell 118: 285–296.

35. Boothby MR, Mora AL, Scherer DC, Brockman JA, Ballard DW (1997) Perturbation of the T lymphocyte lineage in transgenic mice expressing a constitutive repressor of nuclear factor (NF)-kappaB. J. Exp. Med. 185: 1897–1907.
36. Ferreira V, Sidenius N, Tarantino N, Hubert P, Chatenoud L, Blasi F, Korner M (1999) *In vivo* inhibition of NF-kappa B in T-lineage cells leads to a dramatic decrease in cell proliferation and cytokine production and to increased cell apoptosis in response to mitogenic stimuli, but not to abnormal thymopoiesis. J. Immunol. 162: 6442–6450.
37. Esslinger CW, Wilson A, Sordat B, Beermann F, Jongeneel CV (1997) Abnormal T lymphocyte development induced by targeted overexpression of IkappaB alpha. J. Immunol. 158: 5075–5078.
38. Attar RM, Macdonald-Bravo H, Raventos-Suarez C, Durham SK, Bravo R (1998) Expression of constitutively active IkappaB beta in T cells of transgenic mice: persistent NF-kappaB activity is required for T-cell immune responses. Mol. Cell. Biol. 18: 477–487.
39. Hettmann T, Leiden JM (2000) NF-kappa B is required for the positive selection of $CD8^+$ thymocytes. J. Immunol. 165: 5004–5010.
40. Lavon I, Goldberg I, Amit S, Landsman L, Jung S, Tsuberi BZ, Barshack I, Kopolovic J, Galun E, Bujard H, Ben-Neriah Y (2000) High susceptibility to bacterial infection, but no liver dysfunction, in mice compromised for hepatocyte NF-kappaB activation. Nat. Med. 6: 573–577.
41. van Hogerlinden M, Rozell BL, Ahrlund-Richter L, Toftgard R (1999) Squamous cell carcinomas and increased apoptosis in skin with inhibited Rel/nuclear factor-kappaB signaling. Cancer Res. 59: 3299–3303.
42. Seitz CS, Lin Q, Deng H, Khavari PA (1998) Alterations in NF-kappaB function in transgenic epithelial tissue demonstrate a growth inhibitory role for NF-kappaB. Proc. Natl. Acad. Sci. USA 95: 2307–2312.
43. Chaisson ML, Brooling JT, Ladiges W, Tsai S, Fausto N (2002) Hepatocyte-specific inhibition of NF-kappaB leads to apoptosis after TNF treatment, but not after partial hepatectomy. J. Clin. Invest. 110: 193–202.
44. Norlin S, Ahlgren U, Edlund H (2005) Nuclear factor-\{kappa\}B activity in \{beta\}-cells is required for glucose-stimulated insulin secretion. Diabetes 54: 125–132.
45. Fridmacher V, Kaltschmidt B, Goudeau B, Ndiaye D, Rossi FM, Pfeiffer J, Kaltschmidt C, Israel A, Memet S (2003) Forebrain-specific neuronal inhibition of nuclear factor-kappaB activity leads to loss of neuroprotection. J. Neurosci. 23: 9403–9408.
46. Brambilla R, Bracchi-Ricard V, Hu WH, Frydel B, Bramwell A, Karmally S, Green EJ, Bethea JR (2005) Inhibition of astroglial nuclear factor kappaB reduces inflammation and improves functional recovery after spinal cord injury. J. Exp. Med. 202: 145–156.
47. Dawn B, Xuan YT, Marian M, Flaherty MP, Murphree SS, Smith TL, Bolli R, Jones WK (2001) Cardiac-specific abrogation of NF-kappa B activation in mice by transdominant expression of a mutant I kappa B alpha. J. Mol. Cell. Cardiol. 33: 161–173.
48. Cai D, Frantz JD, Tawa Jr NE, Melendez PA, Oh BC, Lidov HG, Hasselgren PO, Frontera WR, Lee J, Glass DJ, Shoelson SE (2004) IKKbeta/NF-kappaB activation causes severe muscle wasting in mice. Cell 119: 285–298.
49. Cai D, Yuan M, Frantz DF, Melendez PA, Hansen L, Lee J, Shoelson SE (2005) Local and systemic insulin resistance resulting from hepatic activation of IKK-beta and NF-kappaB. Nat. Med. 11: 183–190.
50. Poynter ME, Irvin CG, Janssen-Heininger YM (2003) A prominent role for airway epithelial NF-kappa B activation in lipopolysaccharide-induced airway inflammation. J. Immunol. 170: 6257–6265.
51. Schmidt-Ullrich R, Aebischer T, Hulsken J, Birchmeier W, Klemm U, Scheidereit C (2001) Requirement of NF-kappaB/Rel for the development of hair follicles and other epidermal appendices. Development 128: 3843–3853.
52. Ren H, Schmalstieg A, Yuan D, Gaynor RB (2002) I-kappaB kinase beta is critical for B cell proliferation and antibody response. J. Immunol. 168: 577–587.
53. Ren H, Schmalstieg A, van Oers NS, Gaynor RB (2002) I-kappa B kinases alpha and beta have distinct roles in regulating murine T cell function. J. Immunol. 168: 3721–3731.
54. Voll RE, Jimi E, Phillips RJ, Barber DF, Rincon M, Hayday AC, Flavell RA, Ghosh S (2000) NF-kappa B activation by the pre-T cell receptor serves as a selective survival signal in T lymphocyte development. Immunity 13: 677–689.
55. Cheng JD, Ryseck RP, Attar RM, Dambach D, Bravo R (1998) Functional redundancy of the nuclear factor kappa B inhibitors I kappa B alpha and I kappa B beta. J. Exp. Med. 188: 1055–1062.
56. Cao Y, Bonizzi G, Seagroves TN, Greten FR, Johnson R, Schmidt EV, Karin M (2001) IKKalpha provides an essential link between RANK signaling and cyclin D1 expression during mammary gland development. Cell 107: 763–775.
57. Lernbecher T, Muller U, Wirth T (1993) Distinct NF-kappa B/Rel transcription factors are responsible for tissue-specific and inducible gene activation. Nature 365: 767–770.
58. Schmidt-Ullrich R, Memet S, Lilienbaum A, Feuillard J, Raphael M, Israel A (1996) NF-kappaB activity in transgenic mice: developmental regulation and tissue specificity. Development 122: 2117–2128.
59. Bhakar AL, Tannis LL, Zeindler C, Russo MP, Jobin C, Park DS, MacPherson S, Barker PA (2002) Constitutive nuclear factor-kappa B activity is required for central neuron survival. J. Neurosci. 22: 8466–8475.
60. Magness ST, Jijon H, Van Houten Fisher N, Sharpless NE, Brenner DA, Jobin C (2004) *In vivo* pattern of lipopolysaccharide and anti-CD3-induced NF-kappa B activation using a novel gene-targeted enhanced GFP reporter gene mouse. J. Immunol. 173: 1561–1570.
61. Millet I, Phillips RJ, Sherwin RS, Ghosh S, Voll RE, Flavell RA, Vignery A, Rincon M (2000) Inhibition of NF-kappaB activity and enhancement of apoptosis by the neuropeptide calcitonin gene-related peptide. J. Biol. Chem. 275: 15114–15121.
62. Carlsen H, Moskaug JO, Fromm SH, Blomhoff R (2002) *In vivo* imaging of NF-kappa B activity. J. Immunol. 168: 1441–1446.
63. Blackwell TS, Yull FE, Chen CL, Venkatakrishnan A, Blackwell TR, Hicks DJ, Lancaster LH, Christman JW, Kerr LD (2000) Multiorgan nuclear factor kappa B activation in a transgenic mouse model of systemic inflammation. Am. J. Respir. Crit. Care Med. 162: 1095–1101.
64. Gross S, Piwnica-Worms D (2005) Real-time imaging of ligand-induced IKK activation in intact cells and in living mice. Nat. Methods 2: 607–614.
65. Beinke S, Ley SC (2004) Functions of NF-kappaB1 and NF-kappaB2 in immune cell biology. Biochem. J. 382: 393–409.
66. Gerondakis S, Strasser A (2003) The role of Rel/NF-kappaB transcription factors in B lymphocyte survival. Semin. Immunol. 15: 159–166.
67. Kim S, La Motte-Mohs RN, Rudolph D, Zuniga-Pflucker JC, Mak TW (2003) The role of nuclear factor-\{kappa\}B essential modulator (NEMO) in B cel. Proc. Natl. Acad. Sci. USA 100: 1203–1208.
68. Jimi E, Phillips RJ, Rincon M, Voll R, Karasuyama H, Flavell R, Ghosh S (2005) Activation of NF-kappaB promotes the transition of large, $CD43^+$ pre-B cells to small, $CD43^-$ pre-B cells. Int. Immunol. 17: 815–825.
69. Bendall HH, Sikes ML, Ballard DW, Oltz EM (1999) An intact NF-kappa B signaling pathway is required for maintenance of mature B cell subsets. Mol. Immunol. 36: 187–195.
70. Chen CL, Singh N, Yull FE, Strayhorn D, Van Kaer L, Kerr LD (2000) Lymphocytes lacking I kappa B-alpha develop normally, but have selective defects in proliferation and function. J. Immunol. 165: 5418–5427.
71. Goudeau B, Huetz F, Samson S, Di Santo JP, Cumano A, Beg A, Israel A, Memet S (2003) IkappaBalpha/IkappaBepsilon deficiency reveals that a critical NF-kappaB dosage is required for lymphocyte survival. Proc. Natl. Acad. Sci. USA 100: 15800–15805.
72. Ishikawa H, Claudio E, Dambach D, Raventos-Suarez C, Ryan C, Bravo R (1998) Chronic inflammation and susceptibility to bacterial infections in mice lacking the polypeptide (p)105 precursor (NF-kappaB1) but expressing p50. J. Exp. Med. 187: 985–996.
73. Karin M, Cao Y, Greten FR, Li ZW (2002) NF-kappaB in cancer: from innocent bystander to major culprit. Nat. Rev. Cancer 2: 301–310.
74. Yamada T, Mitani T, Yorita K, Uchida D, Matsushima A, Iwamasa K, Fujita S, Matsumoto M (2000) Abnormal immune function of hemopoietic cells from alymphoplasia (aly) mice, a natural strain with mutant NF-kappa B-inducing kinase. J. Immunol. 165: 804–812.
75. Kaisho T, Takeda K, Tsujimura T, Kawai T, Nomura F, Terada N, Akira S (2001) IkappaB kinase alpha is essential for mature B cell development and function. J. Exp. Med. 193: 417–426.
76. Ishikawa H, Carrasco D, Claudio E, Ryseck RP, Bravo R (1997) Gastric hyperplasia and increased proliferative responses of lymphocytes in mice lacking the COOH-terminal ankyrin domain of NF-kappaB2. J. Exp. Med. 186: 999–1014.

77. Grech AP, Amesbury M, Chan T, Gardam S, Basten A, Brink R (2004) TRAF2 differentially regulates the canonical and noncanonical pathways of NF-kappaB activation in mature B cells. Immunity 21: 629–642.
78. Kuhn R, Schwenk F, Aguet M, Rajewsky K (1995) Inducible gene targeting in mice. Science 269: 1427–1429.
79. Perkins ND (2003) Oncogenes, tumor suppressors and p52 NF-kappaB. Oncogene 22: 7553–7556.
80. Vallabhapurapu S, Ryseck RP, Malewicz M, Weih DS, Weih F (2001) Inhibition of NF-kappaB in T cells blocks lymphoproliferation and partially rescues autoimmune disease in gld/gld mice. Eur. J. Immunol. 31: 2612–2622.
81. Hettmann T, DiDonato J, Karin M, Leiden JM (1999) An essential role for nuclear factor kappaB in promoting double positive thymocyte apoptosis. J. Exp. Med. 189: 145–158.
82. Tato CM, Villarino A, Caamano JH, Boothby M, Hunter CA (2003) Inhibition of NF-kappa B activity in T and NK cells results in defective effector cell expansion and production of IFN-gamma required for resistance to Toxoplasma gondii. J. Immunol. 170: 3139–3146.
83. Esslinger CW, Jongeneel CV, MacDonald HR (1998) Survival-independent function of NF-kappaB/Rel during late stages of thymocyte differentiation. Mol. Immunol. 35: 847–852.
84. Senftleben U, Li ZW, Baud V, Karin M (2001) IKKbeta is essential for protecting T cells from TNFalpha-induced apoptosis. Immunity 14: 217–230.
85. Schmidt-Supprian M, Tian J, Grant EP, Pasparakis M, Maehr R, Ovaa H, Ploegh HL, Coyle AJ, Rajewsky K (2004) Differential dependence of $CD4^+CD25^+$ regulatory and natural killer-like T cells on signals leading to NF-kappaB activation. Proc. Natl. Acad. Sci. USA 101: 4566–4571.
86. Sivakumar V, Hammond KJ, Howells N, Pfeffer K, Weih F (2003) Differential requirement for Rel/nuclear factor kappa B family members in natural killer T cell development. J. Exp. Med. 197: 1613–1621.
87. Ogasawara J, Watanabe-Fukunaga R, Adachi M, Matsuzawa A, Kasugai T, Kitamura Y, Itoh N, Suda T, Nagata S (1993) Lethal effect of the anti-Fas antibody in mice. Nature 364: 806–809.
88. Nowak M, Gaines GC, Rosenberg J, Minter R, Bahjat FR, Rectenwald J, MacKay SL, Edwards III CK, Moldawer LL (2000) LPS-induced liver injury in D-galactosamine-sensitized mice requires secreted TNF-alpha and the TNF-p55 receptor. Am. J. Physiol. Regul. Integr. Comp. Physiol. 278: R1202–R1209.
89. Lehmann V, Freudenberg MA, Galanos C (1987) Lethal toxicity of lipopolysaccharide and tumor necrosis factor in normal and D-galactosamine-treated mice. J. Exp. Med. 165: 657–663.
90. Leist M, Gantner F, Bohlinger I, Germann PG, Tiegs G, Wendel A (1994) Murine hepatocyte apoptosis induced *in vitro* and *in vivo* by TNF-alpha requires transcriptional arrest. J. Immunol. 153: 1778–1788.
91. Doi TS, Marino MW, Takahashi T, Yoshida T, Sakakura T, Old LJ, Obata Y (1999) Absence of tumor necrosis factor rescues RelA-deficient mice from embryonic lethality. Proc. Natl. Acad. Sci. USA 96: 2994–2999.
92. Grivennikov SI, Tumanov AV, Liepinsh DJ, Kruglov AA, Marakusha BI, Shakhov AN, Murakami T, Drutskaya LN, Forster I, Clausen BE, Tessarollo L, Ryffel B, Kuprash DV, Nedospasov SA (2005) Distinct and nonredundant *in vivo* functions of TNF produced by T cells and macrophages/neutrophils: protective and deleterious effects. Immunity 22: 93–104.
93. Yamada Y, Kirillova I, Peschon JJ, Fausto N (1997) Initiation of liver growth by tumor necrosis factor: deficient liver regeneration in mice lacking type I tumor necrosis factor receptor. Proc. Natl. Acad. Sci. USA 94: 1441–1446.
94. Iimuro Y, Nishiura T, Hellerbrand C, Behrns KE, Schoonhoven R, Grisham JW, Brenner DA (1998) NFkappaB prevents apoptosis and liver dysfunction during liver regeneration. J. Clin. Invest. 101: 802–811.
95. Lieber A, He CY, Meuse L, Schowalter D, Kirillova I, Winther B, Kay MA (1997) The role of Kupffer cell activation and viral gene expression in early liver toxicity after infusion of recombinant adenovirus vectors. J. Virol. 71: 8798–8807.
96. Hellerbrand C, Jobin C, Iimuro Y, Licato L, Sartor RB, Brenner DA (1998) Inhibition of NFkappaB in activated rat hepatic stellate cells by proteasome inhibitors and an IkappaB super-repressor. Hepatology 27: 1285–1295.
97. Fausto N (2000) Liver regeneration. J. Hepatol. 32: 19–31.
98. Pikarsky E, Porat RM, Stein I, Abramovitch R, Amit S, Kasem S, Gutkovich-Pyest E, Urieli-Shoval S, Galun E, Ben-Neriah Y (2004) NF-kappaB functions as a tumour promoter in inflammation-associated cancer. Nature 431: 461–466.
99. Rupec RA, Jundt F, Rebholz B, Eckelt B, Weindl G, Herzinger T, Flaig MJ, Moosmann S, Plewig G, Dorken B, Forster I, Huss R, Pfeffer K (2005) Stroma-mediated dysregulation of myelopoiesis in mice lacking I kappa B alpha. Immunity 22: 479–491.