Experimental Hematology

Experimental Hematology 2016;44:519–527

REVIEW

The NF-κB family: Key players during embryonic development and HSC emergence

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(Received 10 March 2016; revised 24 March 2016; accepted 26 March 2016)

The nuclear factor-κB (NF-κB) family is a crucial transcription factor group known mainly for its role in the regulation of the immune system and its response to infection in vertebrates. The signaling pathway leading to NF-κB activation and translocation to the nucleus to exert its function as a transcription factor is well conserved among Kingdom Animalia, which has helped to elucidate other roles that NF-κB plays in other biological contexts such as developmental biology. The manipulation of NF-κB members in a diverse range of animal models results in severe developmental defects during embryogenesis, very often leading to embryonic lethality. Defects include dorsal–ventral patterning and limb, liver, skin, lung, neural, notochord, muscle, skeletal, and hematopoietic defects. Here, we recapitulate the research that has been done to address the role that NF-κB plays during embryonic development, in particular to emphasize its recently discovered role in the specification of hematopoietic stem cells (HSCs), the foundation of the hematopoietic system in vertebrates.

The nuclear factor-κB (NF-κB) family is a group of transcription factors found in nearly all cell types. It is evolutionary conserved and present in animals ranging from the fruit fly (*Drosophila melanogaster* [1,2]) to *Homo sapiens* [3]. Since its discovery 30 years ago by Ranjan Sen and David Baltimore [4], this transcription factor family has been found to play key functions in many biological processes, including development, regulation of immune responses [5], inflammation, and wound healing [6]. Dysregulation of the NF-kB pathway has been linked to cancer [6], autoimmune diseases [7,8], and chronic inflammation [9].

In mammals, the NF-κB family is composed of five members: p65 (also termed RelA), RelB, Rel (also termed c-Rel), and the precursor proteins NF-κB1 (p105) and NF-κB2 (p100), which are processed into their active forms, p50 and p52, respectively. These transcription factors generally work as homodimers or heterodimers, and once translocated to the nucleus, they induce or repress specific gene expression by binding to the classical κB sites [10] or to noncanonical sequences [11]. DNA binding and dimerization are triggered by the amino-terminal Rel homology domain, a 300-amino-acid domain shared by all NF-κB proteins [3]. There are two main signaling pathways that can activate NF-κB in mammals. These two pathways differ in the receptors able to trigger NF-κB activation, the downstream intermediate elements, and the NF-κB members that are subsequently activated [12] (Fig. 1). Typically, in the canonical pathway, the heterodimers p50 and p65 are retained in the cytoplasm through their interaction with the nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor alpha (IκBα) proteins (Fig. 1A). IκBα is marked for degradation and released from the p50/p65 complex when phosphorylated by the inhibitor of NF-κB kinase subunit beta (IKKβ, also termed IKK2), which previously has been activated by phosphorylation through a downstream effector of one of the canonical NF-κB receptors (Fig. 1A). IκBα dissociation from the p50/p65 complex allows the former proteins to translocate to the nucleus and

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0301-472X/
http://dx.doi.org/10.1016/j.exphem.2016.03.010

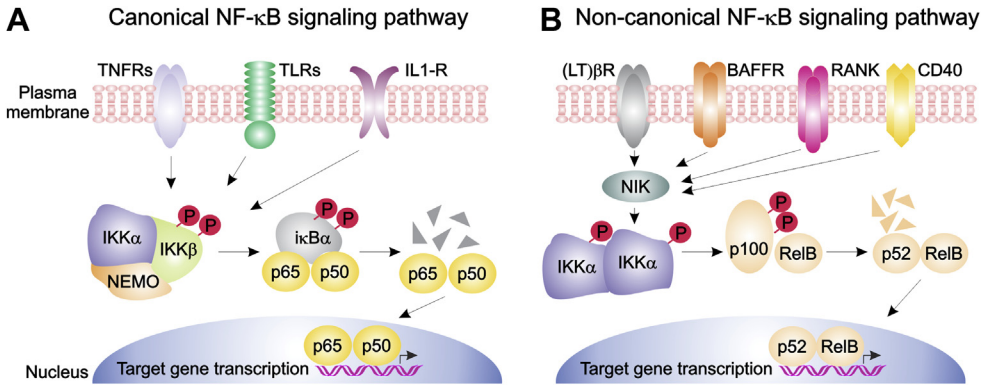

Figure 1. Canonical versus noncanonical activation of NF-κB pathway and its role during development. Briefly, in the canonical NF-κB pathway **(A)**, the activation of canonical NF-κB receptors TNFRs, IL-1R, TLRs, as well as TCR and BCR (not represented here), by their respective ligands converge into IKKβ activation. Activated IKKβ phosphorylates IκBα on serine residues S32 and S36, which will lead to its separation from the p65/p50 complex and degradation. Free p65/p50 heterodimers can then translocate to the nucleus and regulate target gene transcription. In the noncanonical NF-κB signaling pathway **(B)**, activation of noncanonical NF-κB receptors, such as (LT)βR, BAFFR, RANK, or CD40, triggers IKKα phosphorylation and activation by NIK. Activated IKKα then phosphorylates p100 on serine residues S866 and S870, leading to p100 polyubiquitination and partial processing to p52. p52/RelB heterotrimers transcolate to the nucleus to regulate specific gene expression. *BAFFR*=B-cell activation factor; *BCR*=B-cell receptor; *CD40* = cluster of differentiation 40; *(LT)βR* = lymphotoxin β–receptor; *RANK* = receptor activator for NF-κB; *TCR* = T-cell receptor; *TLR* = toll-like receptor; *TNFR* = tumor necrosis factor receptor.

exert their functions as transcription factors (Fig. 1A). In contrast, the noncanonical NF-κB pathway typically involves the heterodimer p100/RelB (Fig. 1B). Similar to the canonical pathway, p100/RelB complexes are held inactive in the cytoplasm until p100 is phosphorylated and partially processed to become the active p52/RelB form, which will translocate to the nucleus to exert its function (Fig. 1B). This process is dependent on NF-κB–inducing kinase (NIK) and NF-κB kinase subunit alpha (IKKα or IKK1) phosphorylation (Fig. 1B).

Although NF-κB function has been studied intensely in the context of the immune and inflammatory response, it is clear that this transcription factor family plays many essential roles during embryonic development (Fig. 2), as evidenced by the developmental abnormalities found in animals and humans with altered NF-κB components.

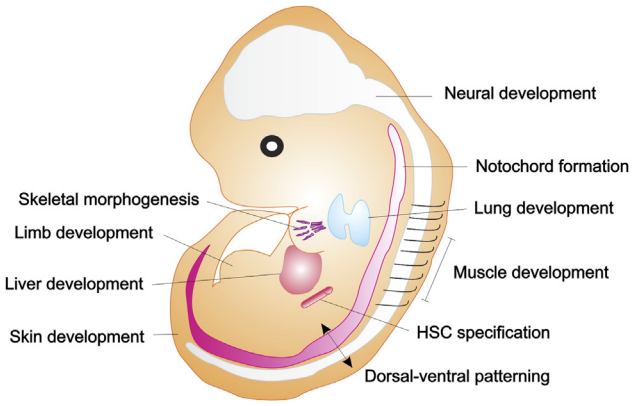

Figure 2. Developmental defects observed during embryogenesis that are associated with manipulation of NF-κB pathway elements. Illustration depicts a vertebrate embryo showing the organs/tissues that are developmentally altered after NF-κB pathway disruption.

There are excellent reviews summarizing the extensive research regarding NF-κB and inflammation/regulation of the immune system. However, to the best of our knowledge, no work has summarized the progress made in the roles of NF-κB in embryonic development. Therefore, in this review, we first focus on the role that NF-κB plays during general embryonic development, including dorsal–ventral patterning; notochord formation; and limb, muscle, lung, skin, neural, and liver development (Fig. 2). Later, we discuss the novel role of this master transcription factor in the development of hematopoietic stem cells (HSCs), the keystone cells of the adult hematolymphoid system.

Roles of NF-κB during embryonic development

The importance of NF-κB during embryonic development is highlighted by the embryonic lethality of several knockout mice with disrupted key components of the NF-κB pathway [13]. These embryonic lethal knockouts include p65 [14], IκBα [15,16], IKKα [17,18], IKKβ [19], and NEMO (NF-κB essential modulator) [20,21]. All mutant mice show developmental defects, dying at early stages of embryonic development. In the following segments, we will synthesize the roles described for NF-κB during embryonic development, not only in mammals, but also in other vertebrate model organisms including zebrafish (*Danio rerio*) and frogs (*Xenopus laevis)*, as well as invertebrates such as *Drosophila*.

NF-κB and embryonic dorsal–ventral (DV) patterning

The contribution of NF-κB to essential developmental processes was indicated clearly from early studies in *Drosophila*. The ancestral NF-κB pathway as observed in *Drosophila* is relatively simple, involving fewer genes

than those present in the vertebrate genome [22]. This relative simplicity has allowed the identification of the biological processes in which NF-κB is essential, bypassing the compensatory effects of other family members that are often present in more complex genomes. Genetic screens in *Drosophila* identified genes belonging to the NF-κB pathway involved in the DV patterning of the embryo. These include the Toll receptor (the counterpart of the mammalian Interleukin 1 receptor [IL-1R]), Tube (functional homolog of the mammalian MyD88 adaptor protein), Pelle (IRAK), Cactus (IκB), Dorsal (the NF-κB homolog), and seven genes upstream of Toll [23]. The dorsoventralization of the embryo is a key process during development in which the ventral and dorsal axes are defined. Depending on the DV position of the cells, these will give rise to different tissues and structures in the embryo. In *Drosophila*, ventral cells yield mesoderm; lateral cells generate ventral ectoderm and the ventral nerve cord; and dorsal cells give rise to dorsal ectoderm. In *Drosophila*, NF-κB is a key regulator of this process. Dorsal activation must exist as a gradient: activated Dorsal (present in the nucleus) must be high in ventral nuclei and absent in dorsal nuclei (in dorsal cells, Dorsal is kept inactive in the cytoplasm) [24]. Embryos lacking Dorsal or Toll are completely dorsalized. These embryos therefore possess cells that develop according to a dorsal fate along the entirely DV axis, giving rise to dorsal ectoderm. In contrast, loss-of-function mutations in Cactus generate completely ventralized embryos [23], with cells all along the DV axis generating mesoderm. Therefore, in *Drosophila*, the DV pattern of the embryo is generated by the specific activation of NF-κB (Dorsal) in a gradient from the ventral to the dorsal side. In cells in which Dorsal is translocated to the nucleus, specific ventral genes, such as *twist*, *snail*, and *rhomboid,* are activated [25–28].

The potential role of the NF-κB signaling pathway in the embryonic DV pattern has also been described in vertebrates, particularly in amphibians and zebrafish. Armstrong et al. showed dorsalization activity in *X. laevis* embryos in response to Dorsal components of the fly, which was inhibited by a dominant Cactus variant [29]. Similarly, overexpression of a dominant-negative form of the murine *Ikbα* in zebrafish to block the NF-κB pathway led to embryonic dorsalization [30]. These experiments suggest that the Dorsal signaling pathway is conserved in the DV patterning of bilateral animals.

NF-κB and notochord formation

The notochord is the defining feature of chordates. It is a structure that provides key signals to pattern the development of the surrounding tissues and serves as a major skeletal element of the developing embryo [31]. The NF-κB pathway has been shown to be essential for ascidian notochord formation [32,33], the most primitive branch of the chordate phylum. The contribution of NF-κB to notochord formation in higher vertebrates has been addressed using the zebrafish model. First, Correa et al. showed that zebrafish NF-κB proteins can be substituted functionally by their mammalian counterparts [30], demonstrating that the NF-κB pathway is therefore conserved in mammals and fish. Overexpression of a dominant-negative form of the murine *Ikbα* in zebrafish to block the NF-κB pathway led to defects in notochord development, generating no-tail embryos [30].

NF-κB and limb development

In 1998, two different papers published in the journal *Nature* showed for the first time that NF-κB is required for normal limb development in the chick [34,35]. Expression of *C-REL* was observed in the wing bud, remaining within the distal mesenchyme as the wing bud matured [34,35]. The investigators then used transdominant-negative mutants of the mouse version of *Ikbα* to block NF-κB activity, resulting in the arrest of limb outgrowth. They noticed that, in these settings, expression of key mesodermal and ectodermal factors involved in limb outgrowth were affected, including *FGF-8*, *MSX-1*, and *LHX-2* [35] as well as *SHH*, *TWIST*, and *BMP-4* [34]. In the mouse embryo, either overexpression of a dominant negative form of *Ikbα* or loss of function of IKKα led to limb development abnormalities [17,18]. However, the investigators found that *Ikkα*-knockout mice had normal activation of the IKK complex and IκBα phosphorylation and degradation in response to the proinflammatory cytokines tumor necrosis factor alpha (TNFα) and IL-1 [17,18], suggesting that canonical NF-κB activation functions normally in these animals. Unlike IKKβ, which is present predominantly in the cytoplasm, IKKα shuttles between the cytoplasm and the nucleus. Within the nucleus, IKKα has been shown to exert kinase-independent functions, modifying gene expression independently of NF-κB (for review, see Huang et al., 2013 [36]). Taken together, these results suggest that an NF-κB–independent role of IKKα is required for limb development during embryogenesis. However, noncanonical NF-κB activation was not evaluated (specifically P52 activation), so another explanation could be plausible in which noncanonical NF-κB activation through IKKα would be necessary for limb development.

NF-κB and muscle development

A role for NF-κB in muscle development first became apparent in *Drosophila* embryos deficient in Toll [37]. These embryos displayed problems in muscle patterning, characterized by deletion, duplication, and errors in muscle insertions in all 30 somatic muscle fibers in the larvae. Mutations in the classical NF-κB components, including *spatzle*, *tube*, and *pelle* generated similar phenotypes [38]. Using mosaic-*Toll*-expressing embryos, Halfon and Keshishian concluded that Toll is required in the epidermis for proper muscle development [38], assigning a nonautonomous role for NF-κB during muscle development. In

contrast, the role of NF-κB in vertebrate myogenesis remains unclear. Most studies have been performed in vitro using mouse myoblast cell lines and have often shown contradictory results. These contradictory results may be due to NF-κB both positively and negatively regulating myogenesis (for reviews, see Bakkar et al. [39] and Mourkioti et al. [40]). It has been proposed that canonical NF-κB signaling is constitutively active in myoblasts, maintaining cells in an undifferentiated state regulated by MYOD, cyclin D1, and YY1. This canonical signal is then postulated to be downregulated once differentiation initiates, and subsequently, the alternative NF-κB pathway is activated during the late myogenic program [41]. Differential activation of each major signaling branch may therefore explain the contradictory results reached thus far. Experiments modulating specific components of each pathway may provide improved precision to our understanding of NF-κB function in mammalian muscle cell development.

NF-κB and liver development

It has been shown that p65-knockout mice die at 15–16 days of gestation due to massive degeneration of the liver by hepatocyte apoptosis [14]. This phenotype can be rescued by mating the $P65^{-/-}$ mice with $Tnf\alpha^{-/-}$ mice, indicating that hepatocyte death is mediated by TNFα [42]. Other evidence of the role of NF-κB on liver development is the severe liver degeneration observed in mice lacking IKKβ [19,43]. Similar to $P65^{-/-}$ mice, $Ikk\beta^{-/-}$ can be rescued after the loss of TNF receptor 1 (TNFR1) function in compound $Tnfr1^{-/-}$, $Ikk\beta^{-/-}$ mice, suggesting that hepatocyte apoptosis is triggered by TNFα. This suggests that canonical activation of NF-κB is a key survival requirement for liver development.

NF-κB and lung development

The first evidence that NF-κB may play a role in lung development was reported by Muraoka et al. [44]. The investigators hypothesized that, because NF-κB had been reported to be a mediator of epithelial-mesenchymal interactions in the developing chick limb [34,35], it could play a similar role in directing branching morphogenesis in the lung, a process similarly based on epithelial–mesenchymal communication. Muraoka et al. showed that *P65* was indeed highly expressed in the mesenchyme adjacent to nonbranching structures of the lung. Using cultures of embryonic chick lungs, they showed that mesenchymal NF-κB repressed epithelial budding, therefore limiting growth and branching of the surrounding epithelium [44]. Expression patterns of *FGF10*, *BMP-4*, and *TGF-β1* (transforming growth factor beta-1), genes that influence lung branching morphogenesis, were altered after NF-κB manipulation, suggesting that NF-κB regulates branching morphogenesis [44]. A role for NF-κB during lung morphogenesis in absence of infection has been also described in mice. *P65* is expressed in the epithelial/periepithelial regions of developing lungs during early embryogenesis in mice [45]. Continuous overexpression of *P65* in the distal pulmonary endothelium increases alveolar types I and II and decreases epithelial apoptosis, enhancing lung maturation [45]. Another study highlighting the function of NF-κB, specifically canonical NF-κB activation, in early alveolar development used the *cre-loxP* system to generate transgenic mice with lung epithelium-specific deletion of *Ikkβ* [46]. These transgenic mice presented increased type II cell apoptosis, decreased epithelial *Vegf* expression, and delayed alveolar formation. Other studies to rule out the role of NF-κB during lung development used immune challenges such as lipopolysaccharide. These conditions, however, are far removed from the relatively sterile environment in which embryos develop, so it is difficult to extract conclusions regarding the physiological role of NF-κB during embryonic development from these studies (for review, see Alvira et al., 2014 [47]).

NF-κB and skin and skeletal development

A key role of the NF-κB pathway during skin development become evident when mice deficient in NF-κB pathway components, including IKKα [17,18,48], IKKβ [49], RELA [14], C-REL [50], RELB [51], and IκBα [16], or mice overexpressing dominant-negative versions of IκB proteins [52] presented with skin abnormalities during development. These skin defects include dysregulated epidermal differentiation caused by aberrant proliferation of cells in the basal layer [15,17,18,48,50], hyperkeratosis and marked epidermal hyperplasia after birth [51], and severe inflammatory skin disease [49]. In addition, both mice and humans with heterozygous *NEMO* mutations develop skin lesions, and these mutations are lethal prenatally in males as an X-linked dominant disorder [21,53,54]. It is important to note, however, that the defects in keratinocyte differentiation described in *Ikkα*-deficient mice seem to be independent of canonical NF-κB activation [55]. Mice deficient in IKKα also present with abnormal skeletal and craniofacial morphogenesis [18,48,55], but these defects seem to be indirectly caused by the lack of IKKα in the skin, because the introduction of IKKα specifically in the basal epidermis of $Ikk\alpha^{-/-}$ mice restores normal skeletal morphology by repressing the expression of fibroblast growth factor 8 (*Fgf8*) [56].

NF-κB and neural development

Neurulation is the embryonic process by which the neural plate folds to give rise to the neural tube, which will form the central nervous system in vertebrates. *Ikkα* and *Ikkβ* single-knockout mouse mutants do not present with neurulation defects. However, because these single-knockout mice could still have partial NF-κB activation [19,48], genetic redundancy of IKKα and IKKβ could mask other roles of these kinases during development. To address this concern, Li et al. generated *Ikkα/Ikkβ*

double-deficient mice and found that, in these animals, the neural tube was unable to close in the hind brain, presumptively due to increased apoptosis of the neuronal epithelium in the hindbrain [57]. In addition, studies in *X. laevis* revealed that Xrel3 (the c-Rel homologous in mammals [58]) modulates the neural patterning elements Shh, Gli1, and Otx2, influencing the anterior neural patterning [59]. Since these initial observations in the late 1990s, it is becoming more and more evident that NF-κB is a major regulator of neural development, learning, and memory (for reviews, see Gutierrez et al. [60], Memet et al. [61], and Crampton et al. [62]). The early embryonic lethality of mice lacking key NF-κB components, such as P65, has made it challenging to characterize these proteins in the development of other tissues past their embryonic lethality. That *Tnfr1*$^{-/-}$/*P65*$^{-/-}$ double-knockout mice are viable has allowed the study of later roles for NF-κB, including the role of p65 in hippocampal neurogenesis [63].

Role of NF-κB in embryonic hematopoiesis

NF-κB has a crucial role in hematopoiesis. The contribution of this transcription factor to the development, differentiation, and homeostasis of the different hematopoietic lineages has been observed from *Drosophila* to humans. *Drosophila* has three different types of blood cells or hemocytes termed crystal cells, plasmatocytes, and lamellocytes. They are mostly produced in the lymph gland during larval stages. Plasmatocytes are the most abundant hemocyte, and they are involved in diverse phagocytic activities; lamellocytes work against parasitic infections. It has been shown that overactivation of the Rel pathway in *Drosophila* leads to high incidence of melanotic tumors due to the aberrant function of lamellocytes forming aggregates around self-tissue in the absence of parasites [64]. In fact, the NF-κB pathway elements Cactus, Toll, Tube, and Pelle were found to be expressed in the nascent hemocytes of the larval lymph gland [65], suggesting that the NF-κB pathway is required intrinsically for hematopoiesis during development. Mutations in Cactus or Toll as well as the constitutive expression of Dorsal lead to hematological defects. Therefore, Cactus mutants contain numbers of hemocytes that have been increased tenfold and enlarged lymph glands, leading to the lethality of these animals during larval stages [65]. In contrast, Toll, Tube, and Pelle mutants present hemocyte deficiency [65]. These observations support a model in which Toll, Tube, Pelle, and Cactus control steady-state hemocyte density in *Drosophila* development.

The central role of the NF-κB pathway in hematopoiesis has also been revealed using genetic approaches in mammals. It is clear that NF-κB plays a pivotal role in the regulation of hematopoiesis in adult mice. Specific NF-κB members are expressed differentially in the different hematopoietic populations and regulate lymphopoiesis, erythropoiesis, and myelopoiesis (for review, see Bottero et al. [66], Gerondakis et al. [67], Denk et al. [68], Zhang et al. [69], and Grossman et al. [70]). In addition, recent studies have shown that both canonical and noncanonical NF-κB activation regulate HSC homeostasis and function intrinsically [71,72]. Despite this evidence showing that NF-κB is essential for adult hematopoiesis in mammals, only a few recent studies have addressed the role of this transcription factor family in the establishment of the hematopoietic system during development. For example, IkBα-deficient mice show increased granulocyte precursors after birth and neonatal lethality due to constitutive NF-κB nuclear activity in these mice [15]. *Relb*-knockout mice also have hematopoietic abnormalities, including myeloid hyperplasia and reduction of erythroid precursors in the bone marrow and a reduced population of thymic dendritic cells [73]. Similar to *Ikbα* mutants, *Relb*$^{-/-}$ mice display a hematopoietic phenotype shortly after birth [73]. Similarities among the NF-κB subunits could mask certain phenotypes in the single mutants because of compensatory effects by other family members. Studies in which two different NF-κB components are depleted have helped to find previously unappreciated roles of the NF-κB pathway during hematopoiesis in development. For instance, whereas REL and RELA are dispensable for hematopoiesis [14,42,74], combined REL and RELA mutations lead to several hematopoietic defects during embryogenesis, including impaired erythropoiesis, deregulated expansion of granulocytes, apoptotic monocytes, and interestingly, fewer fetal HSCs able to reconstitute irradiated recipient mice [70]. Other examples are the impaired lymphoid development in *P50/P65*-deficient mice [75] and defective thymic and splenic architecture and osteoclast and B-cell-development aberrations in *P50/P52*-deficient mice [76,77].

Role of NF-κB in emergence of HSCs, the founders of the adult hematopoietic system

HSCs are a rare population of cells defined by their ability to self-renew and give rise to all blood cells in the vertebrate organism [78]. Interestingly, these cells arise de novo in the embryo during a short period of time and come from a specific endothelial population in the floor of the dorsal aorta (DA) [79–82]. Due to the ability of HSCs to reconstitute the entire blood system of an organism, these cells are used to treat hematological disorders, such as leukemia, by HSC transplantation therapy (HSCT). However in certain HSCT transplantations, HSC content may be insufficient for reliable engraftment, specifically when using umbilical cord blood as the HSC source when the recipient is of adult size, which can lead to failure of engraftment and hematopoietic repopulation in the patient [83]. Extensive research is therefore being performed by numerous laboratories to try to elucidate the key pathways involved in HSC specification. The ultimate goal is to use this knowledge to develop protocols to create HSCs de novo from human pluripotent precursor cells or

to expand purified human HSCs ex vivo. Both approaches have had little success due to our lack of understanding of the native mechanisms used by the embryo to generate HSC fate. Aside from the previous evidence that NF-κB signaling regulates the emergence of the hematopoietic system in *Drosophila* and adult HSC homeostasis and function in vertebrates, until recently, no studies had addressed potential roles for NF-κB in vertebrate HSC specification. Using the unique advantages offered by the zebrafish regarding in vivo visualization of embryogenesis, our group demonstrated that canonical NF-κB activation is required for HSC specification during embryogenesis [84]. Using an NF-κB reporter animal [85], we described discrete cells within the DA experiencing NF-κB activation at the time of HSC specification [84]. The specific endothelial expression of a dominant-negative version of *ikbaa* that impairs NF-κB translocation to the nucleus allowed us to demonstrate that NF-κB activation is required intrinsically within hemogenic endothelium for HSC emergence. These data were later confirmed independently by He et al. using a similar approach [86]. We and others also identified in zebrafish the classical NF-κB activator Tnfa (acting through its receptor Tnfr2) as one of the receptors triggering NF-κB activation in the hemogenic endothelium to specify HSCs [84,86] (Fig. 3). In addition, Tlr4 (acting through Myd88) and Gcsfr have also been identified upstream of NF-κB for HSC specification [86] (Fig. 3). It seems reasonable that, in contrast to *Drosophila* (in which a unique receptor activates NF-κB), different receptors, such as the described Tnfr2, Tlr4, and Gcsfr, activate NF-κB during development in vertebrates. It is well known that Notch signaling is required intrinsically and extrinsically during HSC emergence [87]. Interestingly, we showed that NF-κB and Notch activation occur within the same cell in the hemogenic endothelium to specify HSCs [84] and proinflammatory signaling, which lead to NF-κB activation, promoted Notch activity [86] (Fig. 3). Conversely, we showed that Tnfr2 activation led to *jag1a* transcription, one of the key ligands participating in Notch activation during HSC development (Fig. 3). Whether *jag1a* expression is activated through NF-κB in the contiguous cell of the newborn HSC is still unclear. In addition, it would be informative to elucidate whether NF-κB activates Notch expression or if it modulates its activity to clarify the relationship between these two major transcription factors. In addition, although both studies show a major role for canonical NF-κB signaling in HSC development, potential contributions of noncanonical NF-κB signaling has not been addressed. It would be appealing to further characterize the NF-κB subunits that are involved in HSC emergence as well as the signaling pathways able to activate each, as this would help us gain a better understanding of NF-κB functions in hemogenic endothelium and apply this knowledge ultimately to instruct HSC fate from human pluripotent precursors. Moreover, Sawamiphak [88] and Li [89] demonstrated in zebrafish and mice that interferon gamma, another proinflammatory cytokine, is needed during HSC specification, in this case downstream of Notch signaling [88] (Fig. 3).

Conclusions and future directions

Until recently, it was not appreciated that the same NF-κB family members known to be key in the orchestration of the adult immune response also play key roles in a variety of developmental events. It is now becoming increasingly clear that NF-κB signaling underlies many important processes during embryogenesis, from neural tube formation to HSC emergence. The minor importance attributed to the NF-κB pathway in mammalian development is in part due to the relatively scarce number of developmental phenotypes observed in single-knockout mutants. This appears largely due to compensatory effects by the greater number of NF-κB subunits and receptors able to activate this pathway compared with the unique Toll–Cactus–Dorsal pathway of simpler genomes such as *Drosophila*. This redundancy has been revealed several times by phenotypes emerging only in double-knockout mice that are imperceptible in the single-mutant animals. In addition, the ability of NF-κB to be activated by a broad diversity of receptors raises the question of whether we might be missing additional

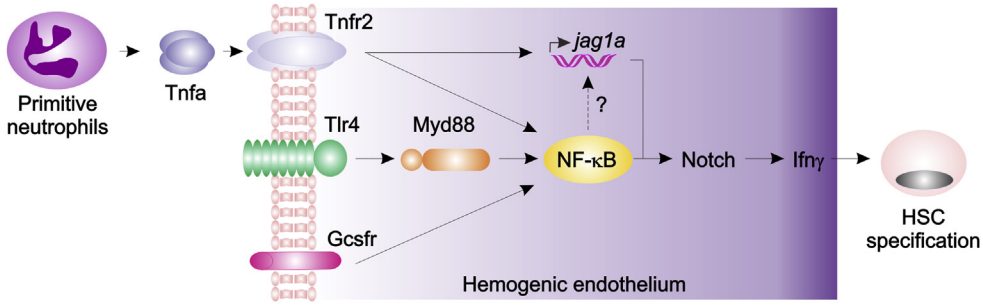

Figure 3. NF-κB is required for HSC specification. Tnfa produced by neutrophils binds Tnfr2 in the hemogenic endothelium to activate NF-κB. NF-κB can also be activated by Gcsfr and Tlr4, the latter through Myd88. Activation of Tnfr2 leads to *jag1a* gene expression, possibly mediated by NF-κB. NF-κB and *jag1a* activate Notch, which leads to HSC specification. Ifnγ, another pro-inflammatory cytokine involved in HSC specification, is downstream of Notch signaling during this process.

developmental roles of this remarkable family of transcription factors due to broader compensatory mechanisms. Further studies done in simpler animal models, such as *Drosophila,* may thus provide valuable information with which to better understand the role of this complex signaling pathway in vertebrate development.

The recent findings reviewed here suggest that the NF-κB pathway might be considered at the level of classical developmental signaling pathways, including Notch, Wnt, bone morphogenetic protein, fibroblast growth factor, Hedgehog, and TGF-β in orchestrating major events in embryogenesis. Whereas some of the developmental defects described after loss of NF-κB components could be attributed to apoptosis of the target tissue, it has become clear that NF-κB functions more broadly than as a simple survival factor. NF-κB orchestrates the expression of key developmental genes involved in ventro-dorsal and neural patterning, lung-branching morphogenesis, and mesodermal and ectodermal lineage factors, among others. The ways in which particular NF-κB homo/heterotrimers are activated to turn on or off these genes may depend on the tissue type or cellular context, and these issues are just beginning to be explored.

Finally, many interesting issues remain to be addressed regarding the roles of NF-κB during development, including the ancestral origins of the NF-κB signaling molecule. For example, its described roles in development could suggest that NF-κB had its origins in regulating developmental events and was later co-opted for use as a stress sensor for infection and inflammation. Another important issue is the identification of the ligand-producing cells that activate NF-κB during these embryonic events. More information concerning the NF-κB signaling pathway during development may help us to better understand new aspects of how it regulates the immune response in adults.

Acknowledgments

This work was supported by the Fundacion Seneca, Agencia Regional de Ciencia y Tecnología de la Región de Murcia (postdoctoral fellowship to Raquel Espín-Palazón).

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