Of mice and men

Meeting on the biology and pathology of NF- κ B

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The third biannual Keystone meeting on nuclear factor- κ B biology and pathology took place in Snowbird, Utah, USA, between 11 and 16 January 2004, and was organized by U. Siebenlist, A. Rao and S. Ghosh

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tions, inflammation and other stressful situations. Activation of this transcription factor is the hallmark of the innate immune response and, owing to the subsequent induction of cytokines, chemokines, specific enzymes and anti-microbial peptides, NF- κ B helps to construct the first line of defence against bacteria and fungi (Silverman & Maniatis, 2001). Furthermore, NF- κ B has an important role in the development of the acquired immune system, mainly as a result of its anti-apoptotic effect (Li & Verma, 2002). Accordingly, loss of NF- κ B function in mice and humans is associated with microbial susceptibility and uncontrolled infections in several organs.

An intense research effort during the past decade has generated a detailed map of the NF- κ B activation pathways (Fig 1). The canonical pathway converges on the I κ B kinase (IKK) complex, which targets the three NF- κ B inhibitory proteins (I κ B α , β and ϵ) for β -TrCP-mediated ubiquitylation and proteasomal destruction. Despite being relatively well-characterized, new aspects of the canonical pathway emerged at the Keystone meeting; for example, the discovery of ELKS, which is a missing component of the IKK complex (Fig 1A), and further clarification of the IKK-independent degradation of I κ B in response to DNA damage (Fig 1C). Perhaps owing to the many inputs that activate NF- κ B, new variations of the main activation theme are still being discovered. A notable example is the 'non-canonical pathway', which culminates in processing of the NF- κ B2 precursor p100 in response to a distinct class of stimulants, particularly in B cells (Fig 1B; Ghosh & Karin, 2002; Dixit & Mak, 2002). Furthermore, it is becoming apparent that NF- κ B activation involves several protein ubiquitylation–deubiquitylation events (and possibly ubiquitylation-like modifications), which regulate IKK and control both the processing and stability of the NF- κ B precursors, and the degradation of all I κ Bs (Fig 1A; Ben-Neriah, 2002).

Although NF- κ B is particularly important during stressful conditions and tissue invasion by hostile microorganisms, it is sometimes overprotective and the host suffers the consequences. At this meeting, as well as making exciting advances in understanding the biology of this transcription factor, we learned a great deal about the pathology that is associated with the dysregulation of NF- κ B, which ranges from skin inflammation and overgrowth to diabetes and cancer.

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Introduction

Few signalling molecules attract sufficient attention to warrant their own scientific field, but one such example is nuclear factor- κ B (NF- κ B). NF- κ B is an inducible dimeric transcription factor that comprises one or two of the five Rel family members: RelA (p65), c-Rel, RelB, NF- κ B1 (p105, which is processed to p50) and NF- κ B2 (p100, which is processed to p52). NF- κ B is expressed ubiquitously, recognizes a common sequence motif, and regulates an exceptionally large number of genes in response to infec-

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Fig 1 | Schematic presentation of canonical (A), non-canonical (B) and DNA-damage-induced (C) NF-κB activation pathways. Proteins are represented as shapes that indicate a functional category according to the Alliance for Cellular Signalling convention (<http://www.signaling-gateway.org>). Most of the abbreviations are defined in the text or can be found online at <http://www.NF-κB.org>.

Structure and signalling

The NF-κB signalling pathway seems to be unique with respect to its rich input source: innumerable membrane and cellular receptors mobilize the pathway. Most NF-κB signals are transduced within the canonical pathway that converges on the IKK complex (Fig 1A); however, some are directed at an ill-defined IKKα-kinase complex (Fig 1B) and others might be streamed through an IKK-independent route (Fig 1C). The mechanism of IKK activation through the canonical pathway is not known and might only be clarified after the determination of the crystal structure of the IKK complex. Z. Chen (Dallas, TX, USA) elaborated on the issue of IKK activation through TRAF6 ubiquitination in response to interleukin 1 (IL-1) stimulation. He showed that the zinc-finger domain of the TAK1 adaptor protein TAB2 selectively binds the Lys63-bonded polyubiquitin chain of TRAF6. This interaction presumably imposes a conformational change on the TAB2-TAK1 complex, which then activates IKK (Fig 1A). It has previously been argued that the lack of an NF-κB phenotype in *Tab2*-knockout mice is hard to reconcile with the ubiquitin activation model. However, Chen maintains that TAB3, which is a homologue of TAB2, can explain this lack of phenotype

because of redundancy between the two molecules. S. Miyamoto (Madison, WI, USA) described a three-step activation pathway of IKK in response to genotoxic stress, which involves the sumoylation of the NF-κB essential modulator (NEMO), its translocation to the nucleus and its subsequent ubiquitylation.

If we thought we knew everything about the canonical IKK complex, I. Verma (La Jolla, CA, USA) showed that we were wrong. He analysed an abandoned 105-kDa protein component that had been purified from the IKK complex by F. Mercurio in 1997. Verma named the protein ELKS and showed that it is an obligatory component of the IKK complex. This coiled-coiled leucine zipper protein probably functions as an IKK scaffold to hold both IKK and IκBα in a single complex (Fig 1A). ELKS is important for IκBα phosphorylation by IKK and is maximally active if trimmed at the amino-terminus. Interference with ELKS expression by small interfering RNA abrogates both IKK activation and the induction of several NF-κB targets. The mechanism of activation of the full-length ELKS remains unclear. Verma raised the possibility that ELKS phosphorylation at the N-terminus might be equivalent to the N-terminal deletion that allows the interaction between IKK and IκBα, and the phosphorylation of the latter.

Is IKK activity essential for NF- κ B activation? Verma argued that it is not, particularly when the activation is triggered by the DNA-damaging agent doxorubicin. This agent mobilizes NF- κ B activation, even in the absence of both IKK α and IKK β , and targets wild-type and phosphorylation-site mutant I κ B α for proteasomal degradation, albeit slowly. These results are in line with the previous studies of M. Karin who showed that carboxy-terminal phosphorylation of I κ B α after DNA damage is possibly regulated by casein kinase II (CKII; Fig 1C). F. Mercurio (La Jolla, CA, USA) implicated IKK in the activation of the related kinase IKK-i(ϵ), which is transcriptionally induced by IKK β and NF- κ B activation. However, in contrast to IKK, IKK-i(ϵ) requires no further activation and is constitutively active. IKK-i(ϵ) knockouts show normal NF- κ B activation, but no induction of a specific set of NF- κ B-dependent proinflammatory genes (for example, *COX2*) in response to lipopolysaccharide (LPS). IKK-i(ϵ) overexpression promotes dimerization and nuclear translocation of interferon regulatory factor 3 (IRF3), induces activation of the DNA-binding protein C/EBP δ and enhances proinflammatory gene induction by transforming growth factor- β (TGF- β). Hence, IKK-i(ϵ) has an important role in regulating inflammation in conjunction with NF- κ B. An unexpected role of the canonical IKK pathway was revealed by S.-C. Sun (Hershey, PA, USA), who showed that LPS activates MEK1 kinase through a complex of the kinase TPL2/Cot and p105. In p105-knockout cells, TPL2 is unstable and LPS fails to activate the MAP kinase pathway, which indicates a crucial role of p105 in stabilizing TPL2.

After its phosphorylation, I κ B α is normally ubiquitinated by the SCF $^{\beta$ -TrCP complex through the substrate-recognition subunit β -TrCP and is targeted for proteasomal degradation (Fig 1A). However, this sequence of events is apparently not universal, and is dependent on the responding cell type and the direction of stimulation. Y. Ben-Neriah (Jerusalem, Israel) reported that in polarized epithelial cells of the colon, basolateral stimulation through Toll-like receptor 9 (TLR9) induces I κ B α degradation and NF- κ B activation. However, apical stimulation has a different outcome: IKK is activated, which leads to I κ B α phosphorylation, but the phosphorylated I κ B α is stable and therefore blocks NF- κ B activation. Furthermore, apical stimulation induces a negative signal that dominates over the positive basolateral signalling, possibly to prevent an inflammatory response to the normal gut microflora. NF- κ B has traditionally been thought to function as a transcription factor via DNA binding. However, G. Ghosh (La Jolla, CA, USA) reported that NF- κ B homo- (p50) and heterodimers can bind specific RNA aptamers with an affinity comparable to DNA (~ 1 μ M). RNA molecules interacting with NF- κ B have no sequence relationship to the cognate DNA target sequence, and the naturally occurring RNA molecules that bind to NF- κ B remain to be identified.

The NFAT transcription factors discussed by L. Chen (Colorado, CO, USA) and A. Rao (Boston, MD, USA) are structurally related to NF- κ B. The NFAT proteins bear a REL-related domain that functions in dimerization, and the overall structure of the NFAT5 dimer is similar to that of the NF- κ B p50 homodimer. NFAT proteins often interact with the AP1 proteins Jun and Fos at promoter sites, where DNA interactions probably stabilize the composite enhanceosome complex. The existence of a large assortment of different NFAT and AP1 proteins, and various conformations of the NFAT dimers, might explain the range of promoter sites that are specifically con-

trolled by the NFAT protein family. On the functional side, Rao described the role of NFAT in a T-cell unresponsive state termed anergy, in which several NFAT-inducible ubiquitin ligases (E3s) cooperate in a process that leads ultimately to the destruction of the immunological synapse between an antigen-presenting cell and a T cell.

Transcription

The liberation of DNA-binding subunits from I κ B proteins is the first decisive level of NF- κ B activation; however, many presentations at this meeting showed that the function of nuclear NF- κ B is subject to other levels of regulation. NF- κ B-mediated transcription is context dependent: distinct κ B sequences contained in promoter regions dictate the specificity and composition of DNA-binding dimers and their associated coactivators. Specific NF- κ B subunits are subject to several post-translational modifications and also contribute to the modulation of chromatin accessibility. Furthermore, the composition of the NF- κ B transcriptosome is dynamic and evolves during the course of a cellular response, which is best illustrated by the temporal exchange of Rel dimers. D. Baltimore (Pasadena, CA, USA) referred to the promoter specificity of NF- κ B in describing a link between a distinct κ B sequence and its relevant NF- κ B dimer and transcriptional coactivator: a single nucleotide exchange in the κ B site of the IP-10 promoter was sufficient to alter the dimer specificity of the promoter, along with the coactivator necessary for IP-10 transcription. S. Ghosh (New Haven, CT, USA) discussed the phenotype of knock-in mice expressing a phosphorylation-deficient RelA Ser 276 point mutant. These animals have a variable phenotype with defects in eye development, which indicates the occurrence of position-effect variegation. In addition, Ghosh described the inhibition of RelA transcriptional activity on its association with histone deacetylase 1 (HDAC1), which was also reported by N. Perkins (Dundee, Scotland). Termination of NF- κ B-dependent gene expression is thought to involve the resynthesis of I κ B α and the nuclear export of NF- κ B, thereby establishing an autoregulatory feedback loop. G. Natoli (Bellinzona, Switzerland) identified a further mechanism that mediates the termination of the NF- κ B response in *I κ B α ^{-/-}* mouse embryonic fibroblasts (MEFs). In these cells, Natoli observed a delayed, yet complete, downregulation of the NF- κ B response, which required polyubiquitylation and proteasomal degradation of DNA-bound RelA. A. Hoffmann (La Jolla, CA, USA) reported that the constitutive NF- κ B activity in MEFs that lack the I κ B family member proteins I κ B α , I κ B β and I κ B ϵ could not be further enhanced by stimulation.

Two presentations discussed the role of the NF- κ B system in chromatin modification and remodelling. The tumour necrosis factor- α (TNF- α)-induced and RelA-dependent recruitment of IKK α to the IL-6 promoter was described by A. Baldwin (Chapel Hill, NC, USA). His experiments indicated that IKK α mediates the cytokine-induced phosphorylation of histone H3 on Ser 10. However, as the canonical NF- κ B activation pathway is intact in IKK α -knockout cells, phosphorylation of Ser 10 is possibly executed by the alternative kinases RSK1, MSK1 and MSK2. The relative contribution of each kinase to this process remains to be determined. F. Shannon (Canberra, Australia) identified a role for nuclear c-Rel in the relocation of a highly positioned nucleosome at the IL-2 promoter. This remodelling correlated with the accumulation of nuclear c-Rel and was absent in *c-Rel^{-/-}* cells.

Neurobiology

Another exiting new aspect of NF- κ B biology is the neurobiology connection. The activation of NF- κ B by inflammatory or stress stimuli is well established, but the investigation of NF- κ B in neurons offers the opportunity to study the regulation of this transcription factor in response to non-pathological signals. Whereas some brain areas show constitutively active NF- κ B, induced NF- κ B activity is observed in hippocampal pyramidal neurons and cerebellar granule neurons in response to the neurotransmitter glutamate and membrane depolarization. The relevance of RelA for synaptic signalling and behaviour has been studied by Baltimore, who showed the importance of the calcium/calmodulin-dependent CaMKII kinase for the constitutive activity of NF- κ B in the brain. The use of GFP-tagged RelA allowed the monitoring of the glutamate-triggered translocation of RelA from synapses to the nucleus, where RelA converts synaptic signals into gene expression. The *in vivo* relevance of these findings was studied in *Rela*^{-/-} mice that were also deficient for the TNF receptor (TNFR1) in order to compensate for RelA lethality. These animals showed normal brain development and anatomy, but behavioural tests revealed a selective deficit in hippocampus-dependent spatial learning. A. Israel (Paris, France) commented on another mouse model in which I κ B α super-repressor (SR) production is regulated by a tetracycline transactivator expressed under the control of a forebrain-specific promoter. In these animals, interference with the entire NF- κ B-dependent transcriptional response in the forebrain is associated with impaired spatial memory, in line with the data obtained from the analysis of *Rela*^{-/-} *Tnfr1*^{-/-} mice. W. Greene (San Francisco, CA, USA) addressed the role of NF- κ B in learning using mice in which I κ B α SR expression is controlled by the tetracycline repressor, the expression of which is driven by the prion protein promoter. Although I κ B α SR expression did not affect several cortical functions, water maze testing in the I κ B α SR mice identified an enhanced capacity for spatial learning, which was associated with increased long-term potentiation and glutamate receptor GluR1 expression in the synaptosomes of hippocampal neurons. These various loss-of-function approaches showed a range of behavioural effects and add a new and interesting dimension to NF- κ B biology.

Innate and adaptive immunity

Innate immunity, which represents a first line of defence against invading pathogens, crucially depends on the rapid activation of NF- κ B. New mouse models with tissue- or cell-specific expression of knock-in alleles of mutated proteins are now providing a more detailed understanding of the individual contribution of the various NF- κ B proteins to immunity. However, many innate immunity components were originally discovered in *Drosophila*. R. Steward (Piscataway, NJ, USA), D. Ferrandon (Strasbourg, France) and S. Wasserman (La Jolla, CA, USA) reviewed the *Drosophila* Imd pathway, which is preferentially induced by Gram-negative bacteria, and the Toll pathway, which is induced by fungi and Gram-positive bacteria. In mammalian cells, NF- κ B activation during innate immunity is initiated by specific patterns of microbial compounds that are recognized by specific receptors, such as TLRs or nucleotide-oligomerization domain/caspase-recruitment domain (NOD/CARD) receptors (Fig 1A). S. Akira (Osaka, Japan) analysed the TLR-mediated signalling pathways and identified MyD88-independent pathways, which use the adaptor proteins TIRAP/Mal and TRIF. *Trif*^{-/-} mice are defective in both TLR3- and TLR4-mediated expression of interferon- β (IFN- β), and in IRF3 activation. Genetic inactivation of the TRIF-related adaptor molecule (TRAM)

revealed defects in cytokine production in response to the TLR4 ligand, but not to other TLR ligands. J.-I. Inoue (Tokyo, Japan) reported on the identification of the adaptor protein TIFA, which activates NF- κ B by linking TRAF6 to IRAK. A database search identified the homologous protein TIFAB, which is an inhibitor of NF- κ B activation. G. Nunez (Ann Arbor, MI, USA) gave an update on NOD proteins. He showed that intracellular mammalian NOD proteins are able to sense and recognize distinct structural motifs in bacterial peptidoglycans, and transmit an IKK-activating signal through the RICK kinase. Significantly, NOD2 mutations that disable the activation of NF- κ B are frequently associated with the occurrence of Crohn's disease, which is an inflammatory bowel disorder. By contrast, mutations that cause constitutive NF- κ B activation are associated with Blau syndrome, which is a familial granulomatosis disease.

Mice deficient in individual NF- κ B DNA-binding subunits have highlighted the importance of this transcription factor for stage-specific B-cell development and survival, as well as for class switching and expression of immunoglobulins. Several talks covered new aspects of the non-canonical NF- κ B activation pathway that is triggered by the TNF family member BAFF, which serves as a fundamental survival factor for B cells. U. Siebenlist (Bethesda, MD, USA) showed that BAFF-mediated processing of p100 to p52 is essential for B-cell development (Fig 1B), as reconstitution of *NF- κ B2*^{-/-} cells with a non-degradable p100 form blocks B-cell development. Siebenlist also showed that the failure of mice that lack the NF- κ B1 (p105/p50) and NF- κ B2 (p100/p52) proteins to progress through the T1 stage of B-cell development can be partially rescued by BCL2 overexpression, which suggests that B-cell development depends on a NF- κ B-mediated prosurvival signal. K. Rajewsky (Boston, MA, USA) used gene targeting to show the importance of the BAFF receptor (BAFFR) for the development of mature B cells. His data indicate that induced deletion of the B-cell receptor only slightly affects BAFFR expression and does not interfere with signalling through the non-canonical activation pathway. X. Li (Cleveland, OH, USA) investigated the role of the adaptor protein Act1/CISK that binds to the BAFFR and to CD40. *Act1*^{-/-} mice develop splenomegaly and lymphadenopathy, and have an increased number of B cells, which is attributable to increased Bcl-X_L expression and survival. *Act1*^{-/-} animals show stronger CD40-induced activation of NF- κ B and the kinases JNK and ERK, which indicates that Act1 might be a negative regulator of B-cell signalling. H.-C. Liou (New York, NY, USA) discussed the reduced viability and impaired cell proliferation in *c-Rel*^{-/-} B cells. The proliferative defect was attributed to impaired CDK2 kinase activity and defective expression of E2F3a, which is necessary for cyclin E expression. Re-expression of Bcl-X_L in *c-Rel*^{-/-} cells restored B-cell survival, but not the proliferative defects.

The role of NF- κ B in T-cell development was discussed by several speakers who used diverse experimental approaches. M. Schmidt-Suppran (Boston, MA, USA) showed that T-lineage-specific ablation of NEMO leads to the absence of peripheral T cells. Conversely, IKK β -deficient mature conventional T cells survive, but the generation of regulatory, natural killer-like and memory-type T cells depends on T-cell receptor (TCR)-mediated activation of NF- κ B through IKK β , Bcl10 and protein kinase-C θ (PKC θ). S. Gerondakis (Victoria, Australia) presented recent data on the role of NF- κ B in two classes of dendritic cells, myeloid and plasmacytoid, the latter of which produce IFN- α in response to viral infections. *p50*^{-/-} *c-Rel*^{-/-} mice have fewer dendritic cells of both classes, however the myeloid, but not the plasmacytoid cells, could be rescued by a *BCL2* transgene. G. Nabel (Bethesda, MD, USA) introduced a new player that regulates NF- κ B

function in T cells. He reported on the MURR1 protein, which inhibits HIV-1 growth in unstimulated CD4⁺ T cells by its ability to inhibit basal and stimulated NF- κ B activity. MURR1 binds to I κ B α and RelA, and regulates I κ B α turnover through effects on proteasomal degradation. J. Moscat (Madrid, Spain) studied the role of atypical PKCs (λ PKC and ζ PKC) by analysing mice deficient for the aPKC inhibitor PAR4 as well as ζ PKC^{-/-} mice. Par4^{-/-} mice showed enhanced NF- κ B activation and increased proliferation of peripheral T and B cells, which was consistent with the phenotype of the ζ PKC^{-/-} mice. Also, the genetic inactivation of the aPKC adaptor p62 revealed a new role for the p62- λ PKC complex in osteoclastogenesis *in vivo*.

Inflammation and chronic diseases

The pro-inflammatory role of NF- κ B is important in the development and perpetuation of chronic diseases, including type II diabetes mellitus. Obesity and a diet high in fat, which are risk factors for diabetes mellitus, are also known to promote chronic low-grade inflammation associated with the increased expression of C-reactive protein (CRP) and IL-6. The role of NF- κ B in this process has been investigated by S. Shoelson (Boston, MD, USA), who analysed the role of NF- κ B in diabetes using a series of mice in which NF- κ B was either activated or inhibited in muscle, liver or fat tissue. Low-level transgene expression of constitutively active IKK β in fat (FIKK) or liver (LIKK) caused insulin resistance in a dose-dependent fashion, whereas liver-I κ B α SR, liver-specific deletion of IKK β or salicylates conferred protection against insulin resistance. LIKK mice showed elevated IL-6 levels, and neutralization of IL-6 reversed the insulin resistance. Siebenlist also showed the involvement of NF- κ B in diabetes, as NF- κ B2^{-/-} mice were significantly protected from high-fat-diet-induced increases in serum glucose and body weight. G. Haegeman (Ghent, Belgium) studied genistein, which is a phyto-oestrogen hormone that inhibits NF- κ B, and showed that the drug interferes with MSK1 recruitment to NF- κ B-regulated promoters and RelA phosphorylation. E. Kieff (Boston, MA, USA) provided an example of an infectious agent that subverts the NF- κ B pathway by presenting evidence for the involvement of IRAK1, TRAF6 and IKK β in NF- κ B activation by the Epstein-Barr virus (EBV) latent-infection membrane protein 1. B. Horwitz (Boston, MA, USA) showed that p50^{-/-} RelA^{+/+} mice are more susceptible to colitis induced by *Helicobacter hepaticus*. This provided further evidence of the important role of NF- κ B in shaping host-pathogen relationships, and of the possibility of NF- κ B-based therapeutic interventions in chronic debilitating diseases.

Cancer

The involvement of NF- κ B in cancer has been inferred from several lines of evidence: Rel-bearing retroviruses in chickens, human Rel translocations, constitutive NF- κ B activity in human tumours and experimental tumour therapy data, mainly in cell lines and human tumour xenografts. Data presented at this meeting extended these observations and also touched, for the first time, on the role of NF- κ B in tumour development. T. Gilmore (Boston, MA, USA) used transactivation-domain mutants to show that the human c-Rel-mediated transformation of chicken lymphoid cells is optimal with intermediate levels of transactivation, and that the c-Rel transactivation domain can be functionally replaced in these transformation assays by a portion of the herpesvirus VP16 transactivation domain. C. Gelinas (Piscataway, NJ, USA) studied the mechanism of transformation by v-Rel in comparison to RelA. Whereas v-Rel transformed primary splenic B cells, neither RelA nor constitutive IKK β transduction

induced B-cell transformation. Transformation required the transactivation domain of v-Rel, whereas the DNA-binding Rel-homology domain of v-Rel could be replaced with that of RelA. G. Sonenshein (Boston, MA, USA) discussed breast-cell transformation by mouse mammary tumour virus c-Rel (MMTV-c-Rel) and MMTV-CK2 transgenes, which yielded slow-growing tumours with increased expression of cyclin D1, Bcl-X_L and c-Myc. V. Bours (Liege, Belgium) identified two phosphorylation sites in Bcl3 and discussed the impact of these phosphorylations on its N-terminal ubiquitylation, gene expression and oncogenic transformation.

Certain types of cancer can be classified according to the NF- κ B status of the tumour. A notable example is the activated B-cell (ABC)-type diffuse large B-cell lymphoma (DLBCL), which was documented by L. Staudt (Bethesda, MD, USA). ABC-type DLBCL is distinguished from other DLBCLs by a specific gene-expression signature composed of several NF- κ B target genes. Five-year survival is significantly lower in ABC lymphoma than in other types, however the ABC lymphoma cells are susceptible to apoptosis by the IKK inhibitor PS1145 (Millennium Pharmaceuticals, Boston, MA, USA) at low (25 nM) concentrations. Interestingly, RelA, but not BCL2, protects against ABC apoptosis, which indicates an active role of NF- κ B in promoting lymphoma survival. Another example of an association between NF- κ B and cancer is Hodgkin's disease (HD). C. Scheidereit (Berlin, Germany) pointed to the universal activation of both AP1 and NF- κ B in Reed Sternberg cells, which are the malignant component of HD, in which the two transcription factors equally contribute to the induction of common HD genes (CCR7, cyclin D2 and Bcl-X_L). HD-cell treatment with the heat-shock protein 90 (HSP90) inhibitors geldanamycin and radicicol resulted in apoptosis, probably owing to the proteasomal degradation of IKK and RIP in the tumour cells.

It seems that NF- κ B has nearly 'reached the bedside', as C. van Waes (Bethesda, MD, USA) reported on early clinical trials of head and neck cancer patients with the proteasome inhibitor bortezomib in conjunction with irradiation. Two out of nine patients showed a reduction in tumour size, nuclear localization of NF- κ B p65 and NF- κ B-modulated cytokine levels in the serum. Another function of NF- κ B activation is in prostate cancer, in which, according to T. Libermann (Boston, MD, USA), many human prostate cancers show signs of NF- κ B activation, particularly during metastasis. Libermann studied the expression of GADD45 isoforms in a prostate cancer-cell line following NF- κ B activation and inhibition. The GADD45 isoforms α and γ are downregulated by NF- κ B activation, whereas isoform β is induced, which results in the inhibition of JNK activation and the repression of apoptosis. Data reported by G. Franzoso (Chicago, IL, USA) provided a mechanistic explanation for the differential effects of GADD45 isoforms in apoptosis, which showed that specific β -isoform residues contribute to inhibiting the JNK activator MKK7, thereby counteracting the pro-apoptotic effects of JNK.

Cylindromatosis, which is a rare hereditary disease that is characterized by the presence of skin adnexal tumours, has illuminated NF- κ B activation from an unexpected angle. The disease develops owing to a loss of the deubiquitylation protein CYLD, which underscores the crucial role of ubiquitylation control in NF- κ B activation. Israel and G. Mosialos (Vari, Greece) reported on the characterization of CYLD, which induces the deubiquitylation of TRAF6, TRAF2 and NEMO, but not of I κ B and β -catenin. In the absence of CYLD, deubiquitylation fails, possibly resulting in sustained NEMO oligomerization and NF- κ B activation. Mosialos showed that CYLD is upregulated in EBV-transformed human B cells, perhaps as a

countermeasure against EBV-induced NF- κ B activation. M. Pasparakis (Rome, Italy) studied NF- κ B in the skin using conditional IKK β - and NEMO-knockouts in the epidermis. The previously proposed role of NF- κ B in keratinocyte proliferation has not been confirmed in this model: both IKK β - and NEMO-deficient mice showed no cell-autonomous hyperproliferation. Skin-specific deletion of NEMO resulted in a phenotype that resembles incontinentia pigmenti. Male mice that lack NEMO in their keratinocytes had thick hard skin with less hair follicles, concomitant with infiltrating granulocytes and massive apoptosis in the epidermis.

Whereas most of the previous data could be interpreted to show the necessity of NF- κ B in tumour maintenance, animal model data presented by M. Karin (La Jolla, CA, USA) and Ben-Neriah provided some insights into the role of NF- κ B in tumorigenesis (that is, the process of tumour development). Karin tested the requirement for NF- κ B activation in dextran sodium sulphate-induced colitis, which is an experimental model of inflammatory bowel disease-associated cancer. IKK β inactivation in epithelial cells or macrophages abrogated the progression of the colitis-associated cancer. However, the mechanism of tumour suppression in the two knockout models was different. Whereas macrophage IKK deficiency curtailed the inflammatory process and consequently the epithelial proliferative response, intrinsic epithelial NF- κ B deficiency resulted in the apoptosis of preneoplastic cells. This could indicate that NF- κ B is not involved in the initiation of inflammation-associated tumours but rather in their promotion and progression. A similar conclusion has been derived from the experiments of Ben-Neriah in a mouse model for hepatitis-associated cancer. *Mdr2*^{-/-} mice, which spontaneously develop hepatitis followed by cancer, were bred with liver-specific I κ B SR transgenic mice. The resulting mice maintained all of the features of hepatitis, but rarely developed tumours. Here again, NF- κ B seems to be a tumour promoter because early tumorigenesis stages were not affected by NF- κ B deficiency. More enigmatic is the role of NF- κ B in a carcinogen-induced liver cancer model: paradoxically, IKK inhibition in this mouse model facilitated the development of hepatocellular carcinoma. Karin maintains that the source of the difference between the inflammation and carcinogenesis models is the enhanced production of reactive oxygen radicals in the latter, which is possibly due to super-activation of JNK in the absence of IKK. Therefore, it is possible that in epithelial cancers, NF- κ B activation has a distinct role in inflammation-associated malignancies and other types of neoplasia.

Synopsis

On the basis of the advances presented at this meeting, can we predict where the field is going? For nearly two decades since its inception, NF- κ B research has progressed in two directions: the first, which is mainly based on molecular studies in cell lines, aims to understand the basis of NF- κ B activation; and the second, which has recently relied on manipulation of the mouse genome, explores the physiological consequences of NF- κ B activation and its dysregulation. Although both strategies are far from being exhausted and our understanding of the basic concepts of NF- κ B activity is still incomplete, there is a growing emphasis on translational NF- κ B research, owing to its tremendous impact on human health and disease. There is little doubt that NF- κ B is a pivotal factor in many, if not all, inflammatory

processes. If we combine the NF- κ B data with recent epidemiological research, there is growing support for a link between inflammation and many chronic diseases. Therefore, further elucidation of the role of NF- κ B in chronic illnesses might provide new insights into the mechanisms of major diseases, such as diabetes, atherosclerosis and cancer. Sooner or later, it will be possible to implement this knowledge into NF- κ B-based therapeutics, probably starting with cancer therapy (Orlowski & Baldwin, 2002). It is likely that at the next Keystone meeting, which will mark the end of the second decade of NF- κ B research, we will learn that the lessons gained in this research field have been successfully translated into clinical practice.

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REFERENCES

- Ben-Neriah Y (2002) Regulatory functions of ubiquitination in the immune system. *Nat Immunol* **3**: 20–26
- Dixit V, Mak TW (2002) NF- κ B signalling. Many roads lead to Madrid. *Cell* **111**: 615–619
- Ghosh S, Karin M (2002) Missing pieces in the NF- κ B puzzle. *Cell* **109** (Suppl): S81–S96
- Li Q, Verma IM (2002) NF- κ B regulation in the immune system. *Nat Rev Immunol* **2**: 725–734
- Orlowski RZ, Baldwin AS (2002) NF- κ B as a therapeutic target in cancer. *Trends Mol Med* **8**: 385–389
- Silverman N, Maniatis T (2001). NF- κ B signalling pathways in mammalian and insect innate immunity. *Genes Dev* **15**: 2321–2342

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