The biology and biochemistry of inflammatory signalosomes

Meeting on Signalling Networks in Immunity and Inflammation

David J. Rawlings

Children's Hospital & Regional Medical Center, Seattle, Washington, USA

The Juan March Foundation symposium on Signaling Networks in Immunity and Inflammation took place in Madrid, Spain, between 22 and 24 May 2005 and was organized by J. Moscat, M. Karin, P. Rennett and C. Martínez-A.

Keywords: adaptor; innate signalling; NF- κ B; TH2 cells

light several new concepts related to these inflammatory signalling cascades.

Initiation and modulation of NF- κ B signalling

The meeting began with an introduction by I. Verma (San Diego, CA, USA) of the events mediating the NF- κ B cascade. The evolutionarily conserved, core NF- κ B signalling module is comprised of five NF- κ B/Rel family transcription factors, five potential I κ B family regulatory subunits and three I κ B kinases (IKKs)—including the catalytically active subunits IKK α and β , as well as the catalytically inactive, co-associated regulatory subunit IKK γ (also known as NEMO; Fig 1; reviewed in Hayden & Ghosh, 2004).

NF- κ B transcription factors form homo- and heterodimers. In unstimulated cells, I κ Bs bind to these NF- κ B dimers, preventing their translocation into the nucleus (through I κ B β), or altering their dynamic partitioning between the nucleus and cytosol to favour cytosolic localization (primarily through I κ B α and ϵ). NF- κ B activation is triggered by a vast number of cell surface or intracellular receptors and is tightly regulated by the IKK complex. There are two known pathways by which cells stimulate the release of NF- κ B from its I κ B tether in the cytosol: the canonical and non-canonical pathways. In the canonical pathway, downstream NF- κ B activation requires phosphorylation of I κ Bs bound to NF- κ B dimers by means of the IKK complex. IKK-dependent phosphorylation then promotes I κ B ubiquitylation and its subsequent degradation by the 26S proteasome. This releases NF- κ B dimers (predominantly p50/c-Rel and p50/p65) to the nucleus where they activate transcription of specific gene programmes. The non-canonical pathway is initiated by a more limited group of receptors—including CD40, lymphotoxin and the B-cell activating factor belonging to the tumour necrosis factor family (BAFF) family receptors. These promote IKK α activation and proteasomal processing of p100, leading to the release of p52/RelB heterodimers. This latter pathway is not discussed in this report.

The NF- κ B pathway is the focus of research in several laboratories and has become increasingly well described. The diverse signals (hundreds described so far) that can trigger this inflammatory cascade highlight its pivotal role in several biological processes. These pathways span innate and adaptive immunity, viral infection, developmental biology and cancer, and are the subject of

EMBO reports (2006) 7, 25–30. doi:10.1038/sj.embor.7400599

Introduction

This two-day workshop on inflammatory signalling was the first Juan March Foundation symposium to be held at the new Cantoblanco campus of the University Autonomous of Madrid. The intimate audience and the breadth of material discussed made for an exciting and highly interactive meeting. Fuelled by excellent wine and strong coffee, scientific conversations continued late into the night after dinner in Madrid's beautiful plazas.

The prominent and long-recognized role of the nuclear factor- κ B (NF- κ B) cascade in inflammatory responses made this pathway a main focus of the meeting. However, emerging paradigms in innate signalling receptors and their effectors—including the Toll-like, Nacht-leucine-rich repeats (NLR) and p53-induced protein with a death domain (PIDD) receptor systems—and the myriad events that are required to fine-tune the adaptive immune response, from inter-chromosomal interaction to specific co-receptors and effectors, also received significant attention. In this report, I attempt to high-

Suite 300, Children's Hospital & Regional Medical Center, 307 Westlake Avenue North, Seattle, WA 98109, USA
Tel: +1 (206) 987 7450; fax: +1 (206) 987 7310;
E-mail: drawling@u.washington.edu

Submitted 14 September 2005; accepted 15 November 2005; published online 16 December 2005

Fig 1 | Members of the nuclear factor- κ B, I κ B and I κ B kinase families. The number of amino acids in each protein is indicated (right). Cleavage sites for p100, p105 and selected phosphorylation and ubiquitylation sites are shown. NF- κ B, nuclear factor- κ B; IKK, I κ B kinase; RHD, Rel-homology domain; TAD, transactivation domain; LZ, leucine zipper; GRR, glycine-rich region; HLH, helix-loop-helix; Z, zinc finger; CC, coiled coil; NBD, NEMO-binding domain; a, α -helical domains. Reproduced with permission from Hayden & Ghosh (2004); see this review for more details and an overview of NF- κ B biology.

intense interest for pharmacological targeting (Karin *et al*, 2004). New data presented at the meeting addressed some surprising commonalities in the core pathway, as well as key mechanisms involved in receptor-specific NF- κ B activation and new paradigms for the coordinated regulation of NF- κ B nuclear activity.

New insights into the canonical cascade. Verma presented surprising new data indicating that IKK1 (IKK α) can act as a negative regulator of the NF- κ B pathway. It achieves this effect by directly sequestering the IKK-associated adaptor, NEMO, from active IKK complexes and thereby limits downstream signalling. *Ikk1*^{-/-} zebrafish show upregulation of NF- κ B-responsive genes and altered development. By contrast, expression of the zebrafish *Ikk1* in mammalian cells leads to the formation of IKK1/NEMO complexes and repression of NF- κ B activity. Verma proposed that, in specific developmental contexts, IKK1 functions predominantly as

a fail-safe system to limit constitutive NF- κ B activation. A similar role, albeit through a distinct mechanism, was recently described for IKK1 in activated macrophages (Lawrence *et al*, 2005).

A principal, unanswered question in the NF- κ B field is whether there is a conserved 'IKK kinase' and the genetic demonstration of this putative activity. S. Ghosh (New Haven, CT, USA) presented data supporting the idea that the TGF-activated kinase 1 (TAK1) might fulfill this essential role. *Tak1*^{-/-}—as well as *Tab1*^{-/-} or *Tab2*^{-/-}—mouse embryonic fibroblasts (MEFs) were generated through SV40 transformation of fibroblasts derived from knockout mice (before embryonic lethality at day 10). Notably, NF- κ B activity induced by both interleukin-1 (IL-1) and tumour necrosis factor- α (TNF α) was abolished in TAK1-knockout cells. This correlated with a loss of IKK phosphorylation and could be rescued through the expression of activated IKK2. Loss of either TAB1 or TAB2 had no effect in these assays despite previous data suggesting a requirement for TNF-receptor-associated factor 6 (TRAF6)-regulated, TAK/TAB ubiquitylation in IKK activation (Kanayama *et al*, 2004). Notably, both Jun amino-terminal (JNK) and p38 activation were also reduced in IL-1-stimulated *TAK1*^{-/-} cell lines. Consistent with this, Ghosh presented data suggesting that a new TRAF6-binding adaptor, ECSIT, might coordinate the co-localization of TAK1, MEK kinase 1/3 (MEKK1/3) and TRAF6 to activate these combined pathways. Conditional *Tak1*-deficient mice will be required to ascertain whether TAK1 functions as a general IKK kinase.

Specific control of immunoreceptor-dependent NF- κ B activation. K. Rajewsky (Boston, MA, USA) presented an overview of his recent work showing the fundamental requirement for the canonical NF- κ B pathway in T- and B-lymphocyte homeostasis. Targeted deletion of either NEMO or IKK2 (IKK β) in B cells, for example, leads to a marked reduction in both the number and lifespan of mature B cells including the follicular, recirculating, marginal zone, and B1a B subsets (Pasparakis *et al*, 2002). This phenotype overlaps with that of targeted deletion of B-cell antigen receptor (BCR) signals in mature B cells (Kraus *et al*, 2004). Rajewsky presented new data showing that mice expressing a constitutively active IKK2 (IKK2EE-knock-in mice) show an increase in I κ B α degradation and nuclear p50, p65, and c-Rel levels, as well as a dosage-dependent increase in the number of resting mature B cells. Consistent with the idea that the BCR is required to generate the constitutive NF- κ B signal that is required for mature cell survival, this allele also overcame the developmental arrest associated with a deficiency in the BAFF receptor, which activates the non-canonical NF- κ B cascade (reviewed in Schneider, 2005). This suggests that BAFF signalling is predominantly a regulator of the number of B cells and that this signal can be fully replaced by constitutive canonical signalling. These observations suggest that the IKK2EE-knock-in might be sufficient to rescue B-cell survival after conditional deletion of the BCR; that is, that the NF- κ B cascade is the paramount constitutive BCR signal.

Activation of protein kinase C (PKC) isoforms strongly promotes NF- κ B activity in lymphocytes. D. Rawlings (Seattle, WA, USA) presented new data that help to explain the long-recognized requirement for PKC in B and T immunoreceptor signalling. The multidomain adaptor protein, caspase recruitment domain (CARD)-MAGUK1 (CARMA1), is also required for immunoreceptor-NF- κ B signals (reviewed in Thome, 2004). Rawlings showed that a linker in CARMA1 binds to PKC isoforms and that phosphorylation of this linker triggers downstream NF- κ B activity.

CARMA1 contains an N-terminal CARD, which also binds strongly to this linker. Linker phosphorylation is predicted to abrogate this autoinhibitory intramolecular interaction, which then triggers a conformational change that allows Bcl10/Malt1 recruitment and assembly of the 'IKK signalosome'. Consistent with this model, deletion of the linker promotes constitutive NF- κ B activation. Locking CARMA1 in its autoinhibited state—through small molecule inhibitors—could provide a new strategy for lymphoid-specific immunosuppression. Additional analysis of the PKC/CARMA1/IKK signalosome should help to determine the events that control IKK activation, including the potential role for TAK1 in this process.

Y-W Choi (Philadelphia, PA, USA) presented some surprising data showing that inducible loss of TRAF6 in T cells leads to a systemic inflammatory disease associated with an apparent reduction in the threshold for T-cell activation. This suggests that the role for TRAFs in T-cell activation is more complex than appreciated at present. Presumably, other TRAF family members must compensate for TRAF6 in the T-cell receptor (TCR)-mediated NF- κ B cascade.

Directing NF- κ B nuclear activity. Several studies have shown elevated nuclear NF- κ B activity in primary tumours and tumour cell lines—for example, this pathway is increased markedly in poor-prognosis B-cell lymphomas (reviewed in Staudt & Dave, 2005). Interestingly, whereas Rajewsky's IKK2EE-knock-in mice show a pronounced increase in peripheral B-cell numbers, this strain did not have overt B-cell lymphomas. Emerging data are providing insight into how alternative nuclear signals function to modulate nuclear NF- κ B activity. Most oncogenic signals coordinate to activate NF- κ B pro-survival pathways, as well as the tumour suppressors p53 and ARF (also known as p19ARF or cyclin-dependent kinase inhibitor 2A). N. Perkins (Dundee, UK) presented data showing that the ARF pathway indirectly opposes NF- κ B (p65) activity (Fig 2). ARF achieves this effect through promoting the activity of the checkpoint kinases, ATM and Rad3-related (ATR) and Chk1. Activated Chk1 is recruited to p65 through an unknown adaptor and phosphorylates Thr 505 in the transactivation domain of p65. In turn, this modification promotes recruitment of histone deacetylase 1 to p65, which attenuates its transcriptional activity. By inhibiting the anti-apoptotic activity of p65, and coordinately enhancing p53 function (through other pathways), ARF efficiently executes its tumour suppressor function. Nuclear modification of p65—on Thr 505 and at least nine other potential regulatory sites—allows various co-signals to modulate p65 activity. These events help to explain the complex outcomes from the integration of NF- κ B signals in alternative lineages and in response to distinct signals.

Verma also presented an interesting analysis of lentiviral transgenic mice expressing p65 fused to the green fluorescent protein. Surprisingly, although there was a basal increase in NF- κ B target genes, p65 remained almost entirely cytoplasmic. This finding held true even in the absence of I κ B α , ϵ , and γ in triple I κ B $^{-/-}$ cells generated using RNA interference (RNAi). This suggests that p65 nuclear binding controls its nuclear localization and p65 interaction with I κ B is not required to maintain its cytoplasmic localization. Cytoplasmic p65 localization probably serves primarily to prevent basal NF- κ B-dependent gene expression and to allow an efficient, inducible recruitment of p65 in response to inflammatory stimuli.

Fig 2 | Model for tumour-suppressor-directed modulation of p65 activity.

Oncogene signalling activates both the nuclear factor- κ B and ARF (also known as p19ARF or cyclin-dependent kinase inhibitor 2A) signalling cascades. ARF promotes Chk1 kinase activity—through the ataxia telangiectasia mutated (ATM) and the ATM and Rad-3 related (ATR) kinase—leading to phosphorylation of p65 at Thr 505. This inhibits p65 transactivation through the recruitment of a histone deacetylase (not shown) and leads to a decrease in B-cell lymphoma apoptosis regulator-XL (Bcl-XL) levels and increased sensitization to apoptotic stimuli. In parallel, ARF promotes p53 activation through the phosphorylation of Ser 15—through the ATR/breast-cancer-associated 1 (BRCA1) complex—leading to cell cycle arrest and apoptosis. Reproduced with permission from Rocha *et al* (2005).

Interplay of NF- κ B inflammatory signals and cancer

An exciting avenue of investigation of NF- κ B signals has been the interplay of this pathway with other inflammatory events that promote carcinogenesis (reviewed in Greten & Karin, 2004). Previous studies in IKK2 $^{-/-}$ models have shown that there is negative feedback between NF- κ B and the JNK signalling cascade and have linked this increased JNK activity to the induction of chronic inflammation. M. Karin (San Diego, CA, USA) presented recent work expanding on this analysis. In mice that specifically lack IKK2 in hepatocytes, NF- κ B signals promoted anti-oxidant signalling. In the absence of IKK2, reactive oxygen species accumulated and promoted sustained JNK activity. This occurred through reactive oxygen species-induced modification of the cysteine residue in the conserved active sites of mitogen-activated protein kinase phosphatases. Such modifications significantly reduce phosphatase activity. In the absence of IKK2, the chemical carcinogen, diethylnitrosamine (DEN), triggered JNK-dependent hepatocyte cell death and the compensatory proliferation of surviving hepatocytes (Fig 3). This necrotic cell death generated the NF- κ B-mediated production of cytokines and growth factors by Kupffer cells, which together led to hepatocyte and tumour outgrowth. These findings suggest that targeting 'inflammatory' pathways in cancer might be either detrimental or beneficial in cases of 'non-inflammatory' solid-tumour carcinogenesis and that such therapeutic effects will depend on both the specific timing and cell types targeted. It will be important to determine the role of specific innate signals in this inflammatory loop.

Innate signalling pathways and inflammatory responses

Pathogen-recognition receptors (PRRs) represent key components of the immune system that trigger both innate effector mechanisms and activation of adaptive immunity. In addition to the prototypic PRRs in the Toll-like receptor (TLR) family, more PRRs have recently been characterized, including the NLR family. Investigation of innate inflammatory signalling is an area of near-exponential growth, and these pathways were the subject of several definitive talks by leaders in this field.

Studies in insect models continue to provide insight into these ancient, highly conserved signalling modules. J. Hoffmann (Strasbourg, France) provided an overview of innate inflammatory signalling pathways (reviewed in Roquet *et al*, 2005), including the recently identified insect peptidoglycan recognition modules. He also presented new data about the interaction of sexual-stage malarial parasites with cells inside the mosquito midgut. Notably, NF- κ B activity in insect cells directly correlated with host susceptibility to infection. Reducing activity of the *Anopheles* rel homologue or RNAi-targeting of the I κ B-like homologue, cactus, both eliminated malarial oocyst development and blocked disease transmission. However, the signals that trigger NF- κ B activation in mosquitoes remain to be defined. Given the global toll of malarial infection, modulation of NF- κ B signals in *Anopheles* mosquitoes might provide a universally important therapeutic target for this pathway in human disease.

B. Beutler (San Diego, CA, USA) highlighted the striking advances made in the past eight years since the positional cloning of the Lps locus in C3H/HeJ mice (reviewed in Beutler, 2005). Using a forward genetic screen based on germline mutagenesis with N-ethyl-N-nitrosourea, his group has identified 12 genes involved in TLR signalling in mice. Recent but still incompletely defined 'hits' include '3d', which is required for TLR3 and TLR9 signalling through an ill-defined phagosome/endoplasmic reticulum docking or fusion function, and 'Feckless', which links TLR4/TRIF signals to the NF- κ B pathway. On the basis of the number of animals evaluated so far and the rate of identification of known TLR pathway genes found using this screen, Beutler estimated that approximately 40 genes will probably encode all of the non-redundant functions in the mammalian Toll pathways. Among the inflammatory signals initiated by TLR engagement is the expression of type I interferons. This pathway is triggered by the recognition of viral and bacterial pathogens through TLRs as well as other intracellular PRRs. On the basis of these observations, a much larger number of gene products will probably be identified in genetic screens assessing regulation of type I interferon production.

Interestingly, H. Wagner (Munich, Germany) showed that mammalian DNA delivered into endosomes using cationic lipids could effectively promote both TLR9-dependent and -independent cell activation. He suggested that differential accessibility to this compartment was a main fail-safe mechanism for cells that might limit aberrant TLR9 signalling. These observations are important in relation to data that suggest that anti-DNA antibodies can promote delivery of mammalian DNA into this compartment, which then leads to B-cell activation in systemic lupus erythematosus (SLE) models.

J. Tschoop (Lausanne, Switzerland) gave an overview of the rapidly expanding, but less understood, NLR family of pattern recognition receptors. The presence of leucine-rich repeats in these proteins, which are also present in the ligand-binding

Fig 3 | I κ B kinase- β links hepatocyte death with cytokine-driven proliferation promoting carcinogenesis. Diethylnitrosamine (DEN) triggers increased cell death in I κ B kinase- β (IKK β) deficient hepatocytes. Necrotic debris, in turn, triggers the NF- κ B cascade in hepatic Kupffer cells. This leads to the release of growth factors that stimulate the proliferation of surviving hepatocytes including those containing DEN-induced mutations. Reproduced with permission from Maeda *et al* (2005).

domains of Toll receptors, suggest that these proteins have complementary roles in intracellular host-microbe interactions. Ligand binding is thought to alter the conformation of the receptors, which triggers oligomerization and downstream signalling (Fig 4). However, the activating signals involved and the key downstream effectors are only beginning to be identified (reviewed in Martinon & Tschopp, 2005). The nucleotide-binding oligomerization domain (NOD) subgroup proteins function through receptor-interacting protein 2 (Rip2) to activate NF- κ B, whereas the Nacht, LRR, and pyrin domain-containing (NALP) subgroup form a high-molecular-weight 'inflammasome' that is responsible for the activation of caspases 1 or 5. Ultimately, both groups mediate the secretion of pro-inflammatory cytokines such as IL-1 β . This signal also triggers TLR-mediated inflammatory responses, which lead to a partial overlap of the activation profile of the innate receptor families. An important role for NLR family genes in human inflammatory disease is highlighted by mutations of NOD2 in Crohn's disease and Blau syndrome. Interestingly, C. McDonald (from G. Nunez's laboratory, Ann Arbor, MI, USA) described Erbin, the first known negative regulator of NOD2. This leucine-rich repeats and PDZ (LAP) family member was identified using mass-spectroscopy analysis of Tap-tagged NOD2 and seems to bind constitutively to NOD2.

Tschoop also presented some exciting new data about the LRR-containing receptor, PIDD. In response to DNA damage, PIDD expression is upregulated and the receptor is recruited into the nucleus. Although its ligands are unknown, PIDD activation triggers 'PIDDosome' assembly and its interaction with the adaptor RIP-associated ICH-1/CED-3 homologous protein with a death domain (RAIDD). This then promotes both NF- κ B and caspase 2 activation.

NF- κ B activation is mediated by means of a complex that includes Rip1, NEMO and TRAF6. Caspase 2 activation promotes a loss in mitochondrial membrane potential and cytochrome *c* (cyt *c*) release. Interestingly, this does not lead immediately to cell death, presumably because of concomitant NF- κ B survival signals. However, conditions of prolonged cell stress tip the balance of these signals and favour apoptosis. Together, these observations suggest that PIDD and caspase 2 are bona fide tumour suppressors.

Notably, PIDD over-expression can promote cyt *c* release in the absence of apoptosis. Survival in this case seems to require a function for Bcl2 (probably indirectly) downstream of mitochondrial damage. In another presentation, T. Mak (Toronto, Canada) showed surprising new work from his laboratory that cyt *c* allele-knock-in mice retain normal electron transfer but fail to activate Apaf1. These data suggest the existence of a cyt *c*- and apoptosome-independent, but Apaf-1-dependent, cell death pathway. Additional work will be required to determine how this alternative pathway fits into our current models of apoptotic death downstream of cyt *c* release.

Modulation of T_H2-type inflammatory responses

The T-helper-cell 1 and 2 (T_H1 and T_H2) pathways, defined by cytokines interferon- γ (IFN- γ) and interleukin-4 (IL-4), respectively, provide distinct CD4 T-cell fates that have important downstream consequences for the host immune response. Notably, T_H2 cells are crucial in the orchestration of allergic inflammatory responses (reviewed in Mowen & Glimcher, 2004) and were an important focal point of the workshop.

Work from several groups has started to identify the regulatory events that control the activation of the T_H1 and T_H2 cytokine loci located on chromosomes 10 and 11, respectively. As part of the T_H2 development programme, several DNAase I hypersensitivity sites have been mapped, including a locus control region (LCR) and the promoters of the cytokine genes (*II3*, *II5* and *II13*) on chromosome 11. Previous studies from R. Flavell (New Haven, CT, USA) also identified large-scale intrachromosomal interactions between these regions in poised chromatin. Flavell presented new data indicating that, in addition to intrachromosomal interactions, chromosomes 10 and 11 show stable interchromosomal interactions—as demonstrated using a chromosome conformation capture, 3C, technique. Naive T cells show a basal interaction between the IFN- γ promoter and T_H2 cytokine hypersensitivity sites. Although not yet demonstrated at the single-cell level, these data suggest that a 'poised mono-allelic state' in resting cells gives way to the intrachromosomal interaction described above. Additional signals may then promote full activation of these loci in differentiating T_H2 cells. The implications of this work probably extend beyond T-helper differentiation, and suggest that cross-chromosomal regulation might coordinate the organization of other genes and might also help to explain the propensity for specific translocation events.

The relative 'strength' and quality of TCR signalling is important in enforcing T_H fate determination. A new player in this arena seems to be the atypical PKC isoform, PKC ζ . Previous studies by J. Moscat (Madrid, Spain) have defined a role for PKC ζ in BCR-triggered NF- κ B signals through its phosphorylation of nuclear p65. Moscat presented data indicating that PKC $\zeta^{-/-}$ mice show reduced T_H2 responses. PKC ζ expression is upregulated during T_H2 differentiation and is required for optimal IL-4 signalling. PKC ζ interacts with Jak1 and phosphorylates its SH2 domain. Consistent

Fig 4 | Nacrt-leucine-rich repeats receptor activation by pathogen-associated molecular pattern ligands. Members of the nacrt-leucine-rich repeats (NLR) family are proposed to exist in an autorepressed conformation that changes upon ligand binding. This promotes assembly of large 'inflammasomes' containing NLR hexamers (or heptamers) complexed with downstream effectors including: caspase 1 or 5 (for NACHT proteins) or receptor-interacting protein (RIP) family kinases [for nucleotide-binding oligomerization domain (NOD) proteins]. These signalling complexes promote secretion of interleukin1 β (IL1 β) and subsequent IL-1R-MyD88-dependent inflammatory signalling (not shown). Activation of p53-induced protein with a death domain (PIDD) occurs in an analogous fashion but mediates both NF- κ B and caspase2 activation. PAMP, pathogen-associated molecular pattern. Reproduced with permission from Martinon and Tschopp (2005).

with these observations, PKC $\zeta^{-/-}$ mice are relatively resistant to ovalbumin-induced allergic airway disease.

Several T-cell co-receptors modulate T_H fate decisions. P. Rennert (Cambridge, MA, USA) presented an overview of his recent work that suggests a role for members of the TIM (T-cell immunoglobulin mucin) protein family in these events (Mariat *et al*, 2005). Intercross analysis of Balb/C and DBA mice initially suggested a role for these transmembrane glycoproteins in T_H2 responses. TIM1 (a homologue of murine Tim2) was originally described as the hepatitis A virus receptor in human cells. Interestingly, TIM1 is a susceptibility gene for atopic disease, but only in individuals exposed to the hepatitis A virus. Conflicting data exist with respect to the expression profile and regulatory role of these proteins. Rennert demonstrated that both murine Tim1 and Tim2 (but not Tim3) are upregulated on T and B cells after ovalbumin challenge. Vanadate treatment promotes an increase in tyrosine phosphorylation of the cytoplasmic tail of TIM1. These data seem consistent with a co-stimulatory role for TIM1 in differentiated T_H cell populations and suggest that this receptor family might be a useful target for immunomodulation. Finally, D. Chaplin (Birmingham, AB, USA) presented cellular studies in an airway asthma model that underscored the important role for both innate and T_H1 effector cells in the recruitment of inflammatory cells. Gr1⁺ myeloid cells, including neutrophils, rapidly enter and transiently accumulate in the lung after allergen challenge. Depletion of this

population markedly reduced both T_H1 and T_H2 recruitment and these events were largely dependent on the metalloproteinase activity of Gr1⁺ cells. T_H1 cells also greatly facilitate T_H2 recruitment into the lung through TNF α and vascular cell adhesion molecule (VCAM)-mediated signals. These findings highlight the interplay of both innate and alternative adaptive signals in so-called ' T_H2 -type' responses. Clearly more work is required to fully understand the regulation of these complex *in vivo* inflammatory responses.

Concluding remarks

The pace and breadth of research in immune cell inflammatory signalling has increased considerably in recent years and this workshop emphasized the rapid progress being made in this arena. These findings underline the biological complexity that must be considered in designing therapeutic interventions to modulate, or harness, these responses in clinical settings. Workshops of this type are crucial to drive the cross-disciplinary interactions that will be required to achieve such goals.

ACKNOWLEDGEMENTS

I thank the speakers for their active participation in this workshop and their comments on this report.

REFERENCES

- Beutler B (2005) The Toll-like receptors: analysis by forward genetic methods. *Immunogenetics* **57**: 385–392
- Greten FR, Karin M (2004) The IKK/NF- κ B activation pathway—a target for prevention and treatment of cancer. *Cancer Lett* **206**: 193–199
- Hayden MS, Ghosh S (2004) Signalling to NF- κ B. *Genes Dev* **18**: 2195–2224
- Kanayama A, Seth RB, Sun L, Ea CK, Hong M, Shaito A, Chiu YH, Deng L, Chen ZJ (2004) TAB2 and TAB3 activate the NF- κ B pathway through binding to polyubiquitin chains. *Mol Cell* **15**: 535–548
- Karin M, Yamamoto Y, Wang QM (2004) The IKK NF- κ B system: a treasure trove for drug development. *Nat Rev Drug Discov* **3**: 17–26
- Kraus M, Alimzhanov MB, Rajewsky N, Rajewsky K (2004) Survival of resting mature B lymphocytes depends on BCR signalling via the Ig α / β heterodimer. *Cell* **117**: 787–800
- Lawrence T, Bebiën M, Liu GY, Nizet V, Karin M (2005) IKK α limits macrophage NF- κ B activation and contributes to the resolution of inflammation. *Nature* **434**: 1138–1143

- Maeda S, Kamata H, Luo JL, Leffert H, Karin M (2005) IKK β couples hepatocyte death to cytokine-driven compensatory proliferation that promotes chemical hepatocarcinogenesis. *Cell* **121**: 977–990
- Mariat C, Sanchez-Fueyo A, Alexopoulos SP, Kenny J, Strom TB, Zheng XX (2005) Regulation of T cell dependent immune responses by TIM family members. *Philos Trans R Soc Lond B Biol Sci* **360**: 1681–1685
- Martinon F, Tschopp J (2005) NLRs join TLRs as innate sensors of pathogens. *Trends Immunol* **26**: 447–454
- Mowen KA, Glimcher LH (2004) Signalling pathways in T_H2 development. *Immunol Rev* **202**: 203–222
- Pasparakis M, Schmidt-Suprian M, Rajewsky K (2002) I κ B kinase signalling is essential for maintenance of mature B cells. *J Exp Med* **196**: 743–752
- Rocha S, Garrett MD, Campbell KJ, Schumm K, Perkins ND (2005) Regulation of NF- κ B and p53 through activation of ATR and Chk1 by the ARF tumour suppressor. *EMBO J* **24**: 1157–1169
- Royet J, Reichhart JM, Hoffmann JA (2005) Sensing and signalling during infection in *Drosophila*. *Curr Opin Immunol* **17**: 11–17
- Schneider P (2005) The role of APRIL and BAFF in lymphocyte activation. *Curr Opin Immunol* **17**: 282–289
- Staudt LM, Dave S (2005) The biology of human lymphoid malignancies revealed by gene expression profiling. *Adv Immunol* **87**: 163–208
- Thome M (2004) CARMA1, BCL-10 and MALT1 in lymphocyte development and activation. *Nat Rev Immunol* **4**: 348–359

David J. Rawlings