REPORT

Use of cell permeable NBD peptides for suppression of inflammation

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Nuclear factor (NF)- κ B is a ubiquitous and essential transcription factor whose dysregulation has been linked to numerous diseases including arthritis and cancer. It is therefore not surprising that the NF- κ B activation pathway has become a major target for development of novel therapies for inflammatory diseases and cancer. However, the indispensable role played by NF- κ B in many biological processes has raised concern that a complete shutdown of this pathway would have significant detrimental effects on normal cellular function. Instead, drugs that selectively target the inflammation induced NF- κ B activity, while sparing the protective functions of basal NF- κ B activity, would be of greater therapeutic value and would likely display fewer undesired side effects. The recent identification and characterisation of the NF- κ B essential modulator (NEMO)-binding domain (NBD) peptide that can block the activation of the I κ B kinase (IKK) complex, have provided an opportunity to selectively abrogate the inflammation induced activation of NF- κ B by targeting the NBD–NEMO interaction. This peptide is synthesised in tandem with a protein transduction domain sequence from *Drosophila antennapedia* which facilitates uptake of the inhibitory peptide into the cytosol of target cells.

Recognition of pathogens by innate or adaptive immune receptors leads to activation of cells displaying these receptors—for example, macrophages, dendritic cells, and lymphocytes. The signal generated by the liganded receptor is communicated to changes in gene expression leading to enhanced expression of effector molecules such as cytokines and adhesion molecules.¹ This process depends on activation of various inducible transcription factors, among which members of the nuclear factor (NF)- κ B transcription factor family play an evolutionarily conserved and critical role.

THE NF- κ B PATHWAY

The wide variety of genes regulated by NF- κ B include those encoding cytokines (for example, interleukin (IL)-1, IL-2, IL-6, IL-12, tumour necrosis factor α (TNF α), lymphotoxin α (LT α), LT β , and granulocyte macrophage-colony stimulating factor), chemokines (for example, IL-8, macrophage inflammatory protein (MIP)-1 α , monocyte chemoattractant protein (MCP)-1, regulated on activation, normal T cell expressed and secreted (RANTES), eotaxin), adhesion molecules (for example, intercellular adhesion molecule-1, vascular cell adhesion molecule, e-selectin), acute phase proteins (for example, serum amyloid A), and inducible effector enzymes (for example, inducible nitric oxide synthase and cyclooxygenase-2). In addition, it has been demonstrated recently that genes encoding evolutionarily conserved antimicrobial

peptides such as β -defensins, are also regulated by NF- κ B. NF- κ B also plays a role in expression of genes encoding molecules important for the adaptive immune response, such as major histocompatibility complex proteins, costimulatory molecules such as B7.1, and cytokines such as IL-2, IL-12, interferon β (IFN β), and IFN γ .¹ The chemokines and cytokines produced in response to NF- κ B activation can stimulate the migration and maturation of lymphocytes. Furthermore, NF- κ B is central to the proliferation and survival of cells mediating the immune response through its ability to activate genes coding for regulators of apoptosis and cell proliferation such as c-inhibitor of apoptosis (IAP)-1, c-IAP-2, A1 (Bfl1), Bcl-X_L, Fas ligand, c-myc, and cyclin D1. This long list of functions suggests that modulation of NF- κ B activity represents an effective therapeutic strategy for combating diseases such as arthritis, asthma, or autoimmunity, that result from dysregulation of otherwise beneficial immune responses. Consequently, there is intense interest in understanding the regulation of this transcription factor in the context of various diseases.

NF- κ B represents a group of structurally related and evolutionarily conserved proteins, with five members in mammals: Rel (c-Rel), RelA (p65), RelB, NF- κ B1 (p50 and its precursor p105), and NF- κ B2 (p52 and its precursor p100).¹ The processing of p105 to p50 is constitutive, whereas the processing of p100 to p52 is regulated. NF- κ B/Rel proteins can exist as homodimers or heterodimers, and although most NF- κ B dimers are activators of transcription, the p50/p50 and p52/p52 homodimers can repress the transcription of their target genes. Among the different members of the Rel family, p65 (RelA) plays a particularly important role since the predominant form of inducible NF- κ B in most cells is the p65:p50 heterodimer. As p50 does not have the ability to drive transcription, it is not surprising that most known regulatory events that impact NF- κ B signalling actually target p65. In resting cells, the majority of NF- κ B/Rel dimers are bound to I κ Bs and retained in the cytoplasm. The I κ Bs are members of a gene family that contains seven known mammalian members, I κ B α , I κ B β , I κ B ϵ , I κ B γ , Bcl-3, and the precursor Rel-proteins, p100, and p105.² Interestingly, p100 serves both as an I κ B and as a specific heterodimeric partner for RelB, such that its processing results in the release of p52:RelB heterodimers. The I κ Bs are characterised by the presence of multiple ankyrin repeats, which are protein–protein interaction domains that interact with the Rel homology domain (RHD) found in the N-terminal half of NF- κ B family members. Cell stimulation with a variety of agonists triggers signal transduction pathways that ultimately result in activation of specific I κ B kinases (IKK). IKK

Abbreviations: CIA, collagen induced arthritis; IL, interleukin; NBD, NEMO binding domain; NEMO, NF- κ B essential modulator; NF- κ B, nuclear factor (NF)- κ B; IKK, I κ B kinases; NKK, NF- κ B inducing kinase; LT, lymphotoxin; nuclear factor (NF)- κ B; TNF, tumour necrosis factor; WT, wild-type

Figure 1 Depiction of the classical and alternative NF- κ B pathways (reprinted with permission from reference 5).

is a complex composed of three subunits: IKK α (IKK1), IKK β (IKK2), and IKK γ (NF- κ B essential modulator (NEMO), IKKAP). IKK α and IKK β are the catalytic subunits of the complex, sharing 52% overall sequence identity, and 65% identity in their catalytic domains.³ The third subunit, IKK γ /NEMO, is the regulatory subunit and is not related to the catalytic subunits.³ In vitro, IKK α and IKK β exhibit similar substrate specificities, targeting two specific serines in the N-terminal regulatory domain of I κ B proteins. However, IKK β is a more potent I κ B kinase than IKK α . By and large, the major stimuli that activate NF- κ B, including byproducts of microbial, fungal, and viral infections and proinflammatory cytokines, also activate IKK. Thus IKK activity is absolutely essential for NF- κ B activation. Phosphorylation of I κ B by IKK tags them for polyubiquitination by a specific ubiquitin ligase belonging to the SCF (Skp-1/Cul/F-box) family.⁴ The actual recognition of N-terminally phosphorylated I κ Bs is carried out by a WD-repeat and F-box containing protein called β -TrCP.⁴ Upon ubiquitination the I κ B proteins are rapidly degraded by the proteasome thereby freeing NF- κ B which then enters the nucleus, binds to DNA, and activates transcription (fig 1). IKK is also involved in phosphorylation induced processing of p100, resulting in the activation of p52:RelB dimers. However, the exact biochemical events that occur upon p100 phosphorylation, and which result in processing, are yet to be fully described.

Signalling to NF- κ B in response to liganding of surface receptors proceeds through either the "classical" or the "alternative" pathway (fig 1). The classical pathway is primarily activated by inducers such as TNF α , IL-1 and lipopolysaccharide (LPS). Activation of this pathway depends on the IKK holocomplex consisting of IKK α /IKK β /NEMO, which phosphorylates I κ Bs to induce their degradation. This pathway is crucial for the activation of innate immunity and inflammation, and for inhibition of apoptosis. The alternative pathway on the other hand is dependent on the protein

kinase NF- κ B inducing kinase (NIK), which phosphorylates and activates complexes of IKK α homodimer.⁵ This pathway is activated by LT β , CD40L, and B lymphocyte stimulator (Blys/BAFF), but not by TNF α , IL-1, or LPS. IKK α selectively phosphorylates p100 and triggers its processing to p52. Because in unstimulated cells, p100 is preferentially associated with RelB, processing of p100 by the alternative pathway leads predominantly to p52:RelB complexes, which bind and transactivate a subset of genes dependent on NF- κ B.⁶ Genetic ablation of components of the alternative pathway, including NIK and IKK α , reveals a selective role for the alternative pathway in B cell maturation, formation of secondary lymphoid organs, adaptive humoral immunity (that is, the production of high affinity antibodies), and production of organogenic chemokines.

NF- κ B IN DISEASE

Studies carried out over the past two decades have helped establish NF- κ B as a key regulator of many biological processes. In general, however, the inducible activity of NF- κ B is most closely linked to the mounting of innate and adaptive immune responses, and as an antiapoptotic, prosurvival factor, which probably helps to prevent killing of immune cells during infection. Aberrant activation of NF- κ B therefore for the most part leads to diseases that are a consequence of dysregulated inflammatory responses or survival. See table 1 for the major human diseases to which NF- κ B has been linked.

IDENTIFICATION OF THE NEMO BINDING DOMAIN OF IKK α AND IKK β

Activation of the IKK complex represents an essential regulatory step in all pathways leading to NF- κ B activation. Although the precise mechanisms through which the IKK complex is regulated are yet to be fully determined, the "core" complex containing the two catalytic subunits, IKK α and IKK β , and the regulatory subunit known as NF- κ B essential modulator (NEMO) or IKK γ , is an indispensable component of all proinflammatory signalling pathways to NF- κ B. To better understand the mechanism by which the IKK complex is regulated, we began to investigate the interaction between IKK β and the NEMO subunit.⁶⁰ These studies revealed that a very small region in the COOH-terminus of IKK α (L738–L743) and IKK β (L737–L742) was essential for stable interaction with NEMO, and for the assembly of the heteromeric IKK–NEMO complex. We termed this region as the NEMO binding domain (NBD). Sequence analysis demonstrated that the IKK β COOH-terminus contains two segments that are homologous to IKK α (denoted a1), a serine-rich domain, and a serine-free region (fig 2A). Analysis of IKK mutants lacking each of these segments indicated that NEMO associates with the COOH-terminus after residue 734 (fig 2A). The region of IKK β from F734–T744 contains a segment that is identical to the equivalent sequence in IKK α , and this region includes the NBD. The IKK sequence then extends for 12 residues forming a glutamate-rich region.

Mutational analysis of the IKK β NBD demonstrated that residues W739 and W741 are critical for NEMO association whereas mutation of the other four amino acids did not affect binding. It was reasoned that the small size of the NBD might permit the design of peptides that could disrupt the interaction of NEMO with the IKKs. Therefore cell permeable NBD peptides were synthesised by fusing a peptide encompassing the NBD to a sequence from the *Drosophila antennapedia* protein that facilitates cellular uptake. Wild-type (WT) NBD peptides inhibited the interaction of IKK β with NEMO in vitro and prevented formation of the endogenous IKK complex in HeLa cells (fig 2B). In contrast,

Table 1 Major diseases in which dysregulation of NF- κ B has been implicated

Disease	Features	References
Atherosclerosis	Atherosclerosis and its clinical manifestations of heart attack, stroke, and peripheral vascular insufficiency are major causes of morbidity and mortality in both men and women. Activated NF- κ B has been identified in situ in human atherosclerotic plaques	7–9
Asthma	Asthma is a chronic inflammation of the bronchial tubes (airways) that causes swelling and narrowing (constriction) of the airways. Increased NF- κ B activity has been observed at key locations in the airways of asthmatic patients and animal models of asthma	10–15
Acquired immune deficiency syndrome	Human immunodeficiency virus (HIV) infection leads to the progressive loss of CD4 ⁺ T cells and the near complete destruction of the immune system in majority of infected individuals. The promoter/enhancer region of the HIV-1 long terminal repeat contains two adjacent NF- κ B binding sites that play a central role in inducible HIV gene expression. High levels of viral gene expression and replication result in part from the activation of NF- κ B, which besides orchestrating the host inflammatory response also activates the HIV-1 long terminal repeat	16–18
Cancer	The ability of NF- κ B to suppress apoptosis and to induce expression of proto-oncogenes such as c-myc and cyclin D1, which directly stimulate proliferation, links it to many forms of cancer. Aberrant, constitutive NF- κ B activity has been detected in many human cancers, including breast cancer, non-small-cell lung carcinoma, thyroid cancer, T or B lymphocyte leukemias, melanoma, colon cancer, bladder cancer, and several virally induced tumours	19–24
Diabetes	Type 1 diabetes, or insulin dependent diabetes mellitus, is a multilateral autoimmune disease characterised by profound destruction of insulin producing cells. Accumulating evidence implicates free radicals and NF- κ B in the destruction of cells and disease progression	25–29
Heart disease	Heart failure is the consequences of many underlying disease states such as hypertension, cardiac hypertrophy, coronary heart disease, arrhythmia, viral myocarditis, and mutations of cytoskeletal protein encoding genes. Strong evidence suggests that the inflammatory response plays a major role in the development of heart disease. Augmented activation of NF- κ B and expression of NF- κ B regulated proinflammatory genes such as TNF α , IL-1, IL-6, IL-8, and inducible nitric oxide synthase have been reported in both experimental models and human patients. These studies collectively suggest that the NF- κ B signalling pathway plays a critical role in the induction and/or manifestation of heart disease	30–34
Muscular dystrophy	Muscular dystrophy is an inherited group of muscle disorders that cause a slow but progressive degeneration of muscles, leading to lifelong pain, disability, and eventual death. In vivo studies targeting skeletal muscles of mdx mice (a mouse model of Duchenne's muscular dystrophy) showed the DNA binding activity of NF- κ B and the expression of NF- κ B regulated inflammatory cytokines such as TNF α and IL-1 starts increasing even before the clinical onset of muscular dystrophy	35–40
Incontinentia pigmenti	For many years no genetic disease caused by NF- κ B dysfunction was known. However, recent reports suggest that mutations in genes of some of the core components of the NF- κ B signalling pathway can cause genetic abnormalities in humans. Incontinentia pigmenti is one such disease of the skin, hair, teeth, and central nervous system. It is an X-linked, dominantly inherited genodermatosis that is antenatally lethal in males	41, 42
Rheumatoid arthritis	NF- κ B has been shown to play diverse roles in the initiation and perpetuation of rheumatoid arthritis. Hyperactivated NF- κ B is a common feature in human rheumatoid arthritis synovium and in various animal models of rheumatoid arthritis, such as adjuvant arthritis in rats, collagen induced arthritis in mice, and streptococcal cell wall induced arthritis in rats	43–47
Alzheimer's disease	NF- κ B immunoreactivity has been observed in early neurological plaques (and surrounding tissue) associated with Alzheimer's disease, whereas more mature plaque types exhibit reduced NF- κ B activity. Several studies have found evidence that amyloid β peptide is able to activate NF- κ B in neurones, thus providing a mechanism by which amyloid may act during the disease pathogenesis of Alzheimer's disease	48–51
Inflammatory bowel diseases	Crohn's disease and ulcerative colitis are common chronic inflammatory bowel diseases that affect up to one million people in the USA. Although the activation of NF- κ B is not causative for inflammatory bowel disease, activated NF- κ B plays a major role in the inflammatory responses in these diseases. High levels of NF- κ B activation have been observed in colonic biopsy samples as well as lamina propria mononuclear cells from patients with Crohn's disease	52, 53
Multiple sclerosis	Multiple sclerosis is a chronic autoimmune disease of the central nervous system in which myelin and myelin-forming cells (oligodendrocytes) become the target of an inflammatory response, resulting in their depletion from multiple sclerosis plaque. The molecular mechanism of oligodendrocyte demise is not known; however, higher levels of TNF α and IL-1, and increased activation of NF- κ B has been observed in active multiple sclerosis lesions	54–56
Inflammatory bone resorption	Bone resorption or osteolysis is a process whereby bone material is lost, often as a result of increased osteoclastic activity. Several lines of evidence have linked NF- κ B to osteoclast formation and bone resorption in response to a range of inflammatory stimuli	57–59

Figure 2 The IκB kinases (IKK) region required for interaction with the nuclear factor (NF)-κB essential modulator (NEMO). (A) Truncation mutants of IKK lacking the extreme COOH-terminus (1-733), the serine-free region (1-707), the serine rich-domain (1-662), and the 1 region (1-644) were used for pull-down analysis by GST-NEMO. None of the mutants interacted with GST. (B) Wild-type (WT) NBD peptides inhibited the interaction of IKKβ with NEMO in vitro and prevented formation of the endogenous IKK complex in HeLa cells. In contrast, mutant peptides (MUT) in which W739 and W741 were substituted with alanine were inactive. (C) Effect of NEMO binding domain (NBD) peptides on NF-κB activity in HeLa cells after tumour necrosis factor α (TNFα) stimulation (from reference 61).

mutant peptides (MUT) in which W739 and W741 were substituted with alanine were inactive. To investigate the effects of the peptides on NF-κB activation, HeLa cells were pretreated with either the wild-type or mutant peptides, prior to stimulation with TNFα. The wild-type NBD peptide inhibited NF-κB activation, whereas the mutant peptide had no effect. Interestingly, treatment with peptide alone (that is, without TNFα) led to a modest (twofold to threefold) activation of NF-κB. It is also important to note that the WT peptide did not completely inhibit NF-κB activity (fig 2C). This suggests that any drug developed to disrupt the interaction of NEMO and IKKβ will most likely leave residual NF-κB activity that might be sufficient to maintain normal cellular processes and prevent spontaneous apoptosis.

USE OF THE CELL PERMEABLE NBD PEPTIDE TO INHIBIT INFLAMMATION IN ANIMAL MODELS

The ability of the cell permeable NBD peptide to suppress NF-κB activity in cells led us to ask whether administration of this peptide to animals would also result in inhibition of NF-κB activity. In our original report describing the NBD peptide, we demonstrated that topical administration of this peptide was able to suppress phorbol 12-myristate 13-acetate (PMA) induced ear oedema, thus demonstrating its efficacy in animals. To better establish the potential efficacy of this peptide in suppressing inflammation in animal models more relevant to human disease, we used two mouse models of inflammation, one using carrageenan to mimic an acute

inflammatory response and a collagen induced arthritis (CIA) model to mimic a chronic inflammatory disease. In the following sections we provide brief summaries of these published studies to illustrate the efficacy of the NBD peptide as an anti-inflammatory drug in animals.⁶²

Effect of NBD peptide in a model of acute inflammation, carrageenan induced mouse paw oedema

Carrageenan injection leads to a time dependent increase in footpad size that peaks at 48 hours and remains detectable 96 hours after challenge (fig 3A). Furthermore, nuclear extracts from soft tissue of each mouse paw injected with carrageenan, collected at different time points after injection (at 12, 48, 72, and 96 hours) reveals significant NF-κB DNA binding activity (fig 3B). NF-κB DNA binding activity was detectable at basal levels in nuclear extracts from tissue of vehicle-alone injected paws, whereas the DNA binding activity was clearly detectable in nuclear extracts from tissue of carrageenan-treated paws at 12 hours reaching a peak at 48 hours, then dissipating to basal level activity by 96 hours. The composition of the NF-κB complex activated by carrageenan was determined to be a classic p50/p65 complex as determined by EMSA supershift analysis (fig 3C). Treatment with WT NBD peptide was found to inhibit oedema formation at 48 hours after carrageenan injection whereas MUT NBD had no discernible effect. As a control the effect of dexamethasone was also studied; this was found to

Figure 3 Time course of mouse carrageenan paw oedema and nuclear factor (NF)- κ B DNA binding activity. (A) Footpad thickness was evaluated at different time points after carrageenan injection. Values are the mean and SEM ($n = 5-25$ mice). (B) Time course analysis of carrageenan-induced NF- κ B activation. Electrophoretic mobility shift assays were performed on nuclear extracts of soft tissue from contralateral uninjected paws (CL) or from carrageenan-injected paws at different time points after injection. Results shown are from one paw in each group representative of four or five paws analysed. (C) Characterisation of carrageenan induced NF- κ B activation using supershift experiments. Nuclear extracts were incubated with antibodies against p65, p50, or c-Rel 30 minutes before incubation with the radiolabelled NF- κ B probe. N = nuclear extract alone (from reference 62).

have the same level of effect as the WT NBD peptide. In contrast, the mutant NBD peptide did not show any effect at any time point. Digital pictures taken 48 hours after carrageenan injection clearly showed oedema in the injected left paw compared with the contralateral, untreated paw. Histologically there was a significant reduction in the level of inflammatory infiltrate, COX-2, and TNF α expression seen in WT NBD treated mice as compared with untreated and MUT NBD treated peptide mice after the challenge (data not shown).

Effect of the NBD peptide on a model of chronic inflammation, CIA

As discussed previously, activation of NF- κ B is associated with the pathogenesis of many chronic inflammatory diseases including asthma, rheumatoid arthritis, and inflammatory bowel diseases. Thus, suppression of NF- κ B is likely to be effective for the treatment of chronic inflammatory diseases. We therefore tested the effect of the NBD peptide on an animal model for rheumatoid arthritis, namely CIA.⁵⁹ Rheumatoid arthritis is characterised by profound inflammation in the joints accompanied by destruction of the joint cartilage and bone. Notably, aberrant NF- κ B activity is strongly associated with many aspects of the pathology of rheumatoid arthritis.

In this CIA model, DBA/1J mice were sensitised with type II collagen in complete Freund's adjuvant. Mice were then subsequently challenged with type II collagen in the presence or absence of wild-type or mutant NBD peptides. Mice treated with wild-type NBD displayed delayed onset, lower incidence, and decreased severity of CIA compared with either untreated or mutant injected arthritic mice (fig 4A). As expected, PBS or mutant NBD peptide injected mice developed severe joint inflammation, as evidenced by marked swelling and erythema of the hind paws and forepaws (fig 4B). Inflammation in the paws of these mice included the ankle joints and extended distally through the limb and digits. In contrast, DBA/1 mice injected with WT NBD peptide were uniformly resistant to CIA and showed only slight visual signs of paw and joint swelling (fig 4B). Importantly, radiographic analysis at the conclusion of the experiment showed markedly less bone and cartilage damage in WT NBD treated mice than in mice treated with either control (PBS) or

mutant NBD (fig 4C). Similarly, measurement of the bone mineral density showed that the wild-type but not the mutant peptide significantly reduced CIA induced bone destruction (fig 4D). Further evidence of the effects of peptide treatment on cartilage and bone destruction was obtained by histological analysis of the knee joints. The cartilage of both the PBS and mutant peptide injected CIA mice showed multiple superficial erosions, with infiltration of polymorphonuclear cells and small lymphocytes into the synovial fluid, accompanied by proliferation of the synovial lining (fig 4E, upper panel). In addition, the surrounding soft tissue in these mice was heavily infiltrated with polymorphonuclear cells, small lymphocytes and macrophages. In contrast, mice injected with wild-type NBD did not develop inflammation, cartilage destruction and bone erosion, and their joints were histologically identical to those of non-CIA control mice. Analysis of the number of osteoclasts present in the joints of the CIA mice showed significant increase in the joints of PBS and mutant NBD treated CIA mice (Figure 4E, lower panel). In contrast, the number of osteoclasts in mice treated with wild-type NBD was no different from that in non-CIA control mice. CIA induced NF- κ B DNA binding activity was absent in the joints of mice injected with WT peptide, and was accompanied by reduced levels of TNF α and IL-1, but increased IL-10. Matrix metalloproteinase 9 (MMP9), an NF- κ B dependent gene that is a marker of activated osteoclasts, was also reduced on treatment with WT-type NBD peptide. Throughout the study, neither overt toxicity or lethality, nor damage to either kidneys or livers, was observed (fig 4F).

POTENTIAL ADVANTAGES OF NBD BASED DRUGS OVER CURRENTLY AVAILABLE DRUGS THAT HAVE ANTI-NF- κ B ACTIVITY

Currently, a large variety of drugs are available exhibiting varying degrees of effectiveness in suppressing NF- κ B. However, few of these compounds are truly NF- κ B specific and hence are likely to affect other pathways. At present many pharmaceutical companies have ongoing programmes to isolate small molecule inhibitors of the IKK kinase. These inhibitors are almost always act by competing for the ATP binding pocket which is shared between all kinases. Despite this potential for cross-reactivity, many of these compounds

Figure 4 The NBD peptide reduces inflammation and suppresses bone destruction in an experimental model of collagen induced arthritis (CIA). (A) Clinical assessment by paw thickness measurement (left) or clinical scoring (right), in CIA mice injected intraperitoneally with PBS, wild-type (WT), and mutant peptides. ○, values from naive non-CIA mice. (B) Paw oedema in naive controls or CIA induced either PBS, WT or MUT NBD mice. (C) Representative knee (left) and ankle (right) joint radiographs show markedly less destruction in CIA mice treated with WT NBD peptide as compared with PBS or MUT NBD peptide. (D) Bone mineral density (BMD) at the knee joints of each experimental group (n=5). (E) Histological analysis of haematoxylin and eosin (H&E) stained (upper) and tartrate resistant acid phosphatase (TRAP) stained (lower) sections of joints from each experimental group. (F) Representative microscopic images of liver and kidney sections from untreated mice and mice injected daily with the WT NBD peptide for 30 days (from reference 59). **p<0.001 as compared with the PBS injected control group.

can be quite specific for individual kinases, and both published and anecdotal reports indicate that a number of such inhibitors have been identified. However, probably indicative of the potential difficulties that such compounds may face in clinical development, are studies with one such

compound from Millennium Pharmaceuticals (Cambridge, MA), which had been characterised as highly specific for IKKβ with no apparent effect on IKKα or another 30 kinases tested.⁶³ A single treatment dose was found to have no effect on the granulocyte population or pre-B and B cells, however, the inhibitor had a marked effect on the bone marrow B cell colony forming unit content suggesting that inhibiting IKKβ with this small molecule leads to a loss of bone marrow B cell progenitor population. Multiple dosing of the inhibitory compound led to a nearly complete loss of B cells, raising serious questions about the therapeutic viability of such strong IKKβ inhibitors. In marked contrast, daily administration of the NBD peptide for up to six weeks was dramatically effective in suppressing multiple types of inflammation, but did not reveal any apparent toxicity on the animals or in the B cells, most likely reflecting the fact that NBD peptide does not affect basal NF-κB activity. In fact the unique advantages of a drug specifically targeting the NBD–NEMO interaction domain can be summarised as shown in table 2.

Although the cell permeable NBD peptide represents a viable candidate for further development as an anti-inflammatory drug, there are without doubt, disadvantages to its use directly as a drug. Chief among them is that peptides have to be administered either intravenously or ip

Table 2 Advantages of drugs targeting the NBD–NEMO interaction	
NBD based drugs	Drugs targeting the kinase domain of IKK
Will <i>not</i> affect basal NF-κB activity	May ablate essential, basal NF-κB activity
Have well defined molecular site of action	Have well defined molecular site of action
Specific for classical NF-κB activation pathway, will <i>not</i> affect NF-κB alternative pathway	Will inhibit both classical and alternative NF-κB pathways
Will <i>not</i> target active domain of kinase, hence unlikely to affect other essential kinases	Potential cross-reactivity with other kinases

thereby removing the potential benefit of an orally available compound. An equally daunting challenge is the cost-of-goods issue. Although larger peptides have been used in the past as drugs (for example, Fuzeon by Trimeris/Roche), and large scale dedicated facilities can synthesise peptides at costs significantly lower than charged by commercial laboratory vendors, it still remains a major hurdle for pharmaceutical companies considering clinical development. Therefore we believe that although NBD peptide represents a viable candidate for further development as an anti-inflammatory drug, identification of a small molecule inhibitor of this interaction is likely to provide more viable candidates for therapeutic development.

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