



Nuclear Factor-Kappa B and Alzheimer Disease, Unifying Genetic and Environmental Risk Factors from Cell to Humans

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Alzheimer's disease (AD) is the most common form of dementia, an irreversible, progressive disease that causes problems with memory, thinking, language, planning, and behavior. There are a number of risk factors associated with developing AD but the exact cause remains unknown. The predominant theory is that excessive build-up of amyloid protein leads to cell death, brain atrophy, and cognitive and functional decline. However, the amyloid hypothesis has not led to a single successful treatment. The recent failure of Solanezumab, a monoclonal antibody to amyloid, in a large phase III trial was emblematic of the repeated failure of anti-amyloid therapeutics. New disease targets are urgently needed. The innate immune system is increasingly being implicated in the pathology of number of chronic diseases. This focused review will summarize the role of transcription factor nuclear factor-kappa B (NF- κ B), a key regulator of innate immunity, in the major genetic and environmental risk factors in cellular, invertebrate and vertebrate models of AD. The paper will also explore the relationship between NF- κ B and emerging environmental risk factors in an attempt to assess the potential for this transcription factor to be targeted for disease prevention.

Keywords: nuclear factor-kappa B, Alzheimer, dementia, cell lines, invertebrate models, rodents, humans

INTRODUCTION

The global burden of dementia is devastating, with an estimated 35 million people affected and the annual cost estimated to exceed \$1 trillion by 2018 (1, 2). Despite greater knowledge of the pathogenic sequelae of disease, repeated failure in drug trials has led to a switch in emphasis from disease treatment to disease prevention (3–5). Alzheimer's disease (AD) is the most common dementia subtype yet no single theory has been able to account for the multiple risk factors leading to the pathological and clinical features (3). The deposition of excess extracellular beta amyloid (A β) protein and subsequent tauopathy has long been felt to be a cause of the disease overshadowing alternative hypotheses including microglial dysfunction, vascular disease, mitochondrial insufficiency, and metabolic disease.

However, the recent failure of another promising anti-amyloid treatment in large phase III trials has dealt a significant blow to the credibility of the amyloid hypothesis (6). The pathognomonic role of A β is now also being questioned with the discovery that A β acts as an antimicrobial peptide (AMP) in cell lines, nematode, and rodent models. A β production following exposure to neurotoxic fungi and bacteria provided significant neuroprotection (7). Amyloid over-production may therefore

be a downstream product of immune dysregulation rather than a disease process in itself. In support of this, several genes are involved in innate immunity are associated with an increase in AD (8) (see **Table 1**). More work is clearly needed to explore the interaction between amyloid and the innate immune system.

Aging is the most significant risk factor for developing AD and recent findings have shown tissue specific brain inflammation, mediated by NF- κ B is associated with aging (12–15). Hypothalamic NF- κ B levels are negligible in young mice and *Drosophila*. Significant activation begins in middle age. The resulting downstream increase in AMPs leads to increased local microglial activity, subsequent decline in gonadotrophins and aging (12, 14). Interestingly, hypothalamic inflammation is relatively higher when compared to neurons and glial cells in other vulnerable brain regions such as the hippocampus (12). In support of this hypothalamic-specific regulation of aging, drugs known to extend lifespan in mice reduce hypothalamic inflammation but have little anti-inflammatory effect on hippocampal neurons (14).

Known inducers of NF- κ B activity are highly variable and include reactive oxygen species (ROS), interleukin 1-beta (IL-1 β), tumor necrosis factor alpha (TNF- α), bacterial lipopolysaccharides (LPS), isoproterenol, and ionizing radiation (16, 17). In addition to stimuli that activate NF- κ B in other tissues, NF- κ B in the nervous system can be activated by growth factors and synaptic transmission such as glutamate (18). These activators of NF- κ B in the nervous system all converge upon the inhibitor of kinase kinase (IKK) complex (**Figure 1**).

Nuclear factor-kappa B transcription factors include a collection of proteins with functions conserved from the fruit fly *Drosophila melanogaster* to rodents and to humans (**Figures 1** and **2**). They are present in all human and most animal cells and regulate the expression of more than 400 genes, including a multitude of inflammatory mediators associated with a variety of chronic inflammatory diseases including cancer, diabetes, and AD (19, 20). Lately, Rel/NF- κ B homologs have also been found to occur even in organisms as simple as *Cnidarians* (e.g., sea anemones and corals), *Porifera* (sponges), and the single-celled eukaryote *Capsaspora owczarzaki*, but are notably absent in yeast and the nematode *Caenorhabditis elegans* (21). Activated NF- κ B regulates the expression of specific genes, including isoforms of SET, directly implicated in the pathogenesis of AD (22). Conversely, expression of the mammalian family of Sirtuin deacetylases, known to attenuate the effects of aging, down regulates NF- κ B (23, 24).

Studies of aging populations have enabled the identification of a number of genetic and environmental risk factors that appear to influence susceptibility to developing AD (25, 26). This paper will review the interaction between these risk factors and NF- κ B, first looking at the major known genetic risk factors in cell, invertebrate, and vertebrate models. It will then focus on the major known environmental risk factors in these models before reviewing emerging environmental risk factors. Finally, the paper will review protective mechanisms across various experimental models and whether their association with NF- κ B.

The repeated failure of disease modifying trials in AD demands that new treatment targets are urgently identified. The recent findings that implicate the innate immune system in AD provides an opportunity to review the evidence for NF- κ B as a key immune system regulator in the prodromal stage in the hope of identifying a target for treatment and prevention.

GENETICS

Neuronal Cell Lines/Human Autopsy Studies

Overexpression of the amyloid precursor protein (APP) gene is associated with familial aggregation of late onset AD and dramatically increases susceptibility to early AD in Down's syndrome. APP is cleaved by the beta-secretase BACE1 into amyloid monomers that form oligomers that eventually become plaques in the brain and vasculature. Both BACE1 and NF- κ B are increased in the brains of AD patients, with NF- κ B directly upregulating BACE1 and the APP gene (27, 28). Medications such as minocycline, that inhibit NF- κ B but not BACE1 or APP, reverse this process (28, 29).

The e4 variant of the APOE gene, which codes for a cholesterol transporting protein, is the largest, single gene risk factor for AD (30). In APOE e4-positive Schwann cell lines, when compared to APOE e3-expressing cells, excess production of IL6, IL10, and nitrous oxide results from a failure to inhibit NF- κ B (31). These findings are replicated in neural cells and fibroblasts from AD patients where APOE e4 acts as a transcription factor responsible for regulating NF- κ B expression (32). Curiously, cells from the somatosensory cortex of AD patients, an area of the brain that is resistant to disease, display upregulation of NF- κ B (33). However, this may reflect the earliest inflammatory hallmark of disease as previous autopsy studies have shown increased NF- κ B activation in evolving A β deposits with a reduction in areas surrounding more mature plaques (19, 33).

TABLE 1 | Emerging genetic risk factors for Alzheimer's disease (AD) and their associated with nuclear factor-kappa B (NF- κ B) and amyloid (β).

Gene implicated in late onset AD	Function	Increased risk of AD	Interaction with NF- κ B	Interaction with amyloid
TREM2	Immunity	Reduced expression increases risk: slight-medium	NF- κ B suppresses hippocampal TREM2 expression (9)	TREM2 required for microglial amyloid clearance (9)
CD33	Immunity	Mild	CD33 activates NF- κ B in myeloid cells	CD33 inhibits microglial A β uptake and clearance (10)
CR1	Immunity	Mild-medium	Microglial CR1 activation associated with increase in NF- κ B (11)	Uncertain (11)
INPP5D	Immunity	Mild	Negative regulator of NF- κ B expression (12)	Uncertain

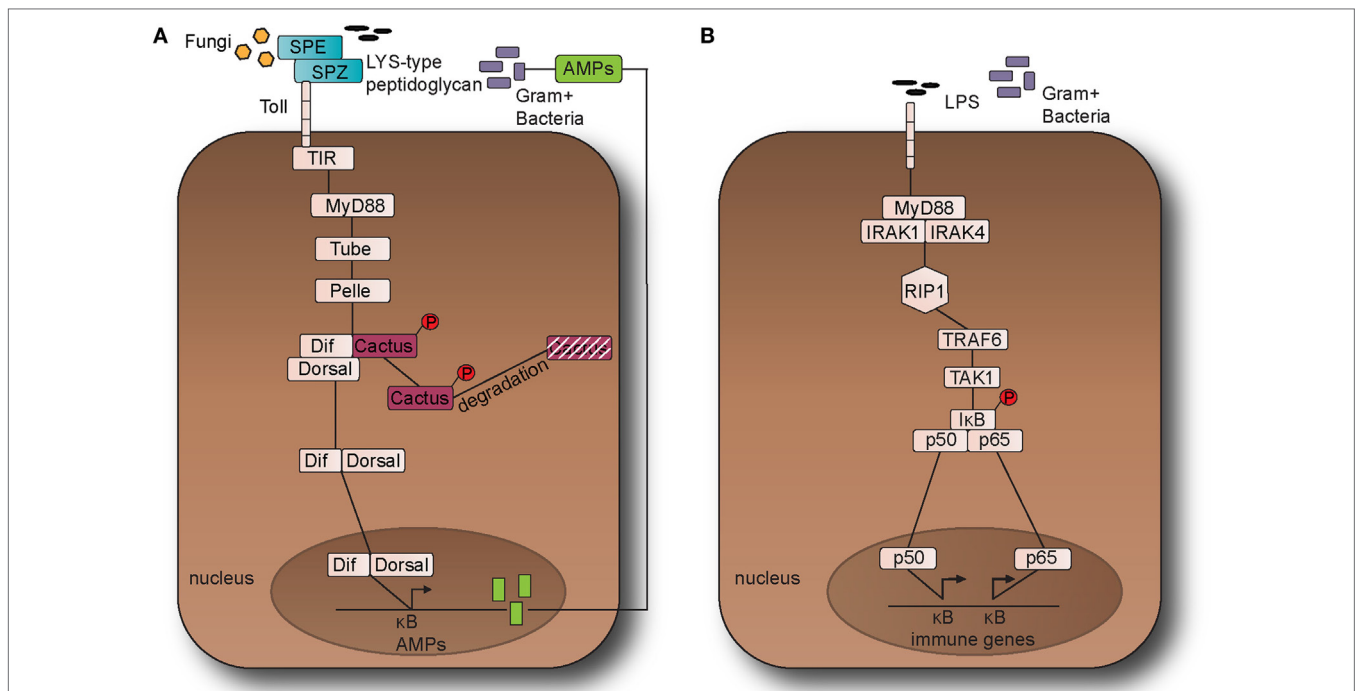


FIGURE 1 | The Toll pathway in fruit fly and the Toll-like receptor (TLR) 4 pathway in the mouse. **(A)** The Toll pathway in *Drosophila melanogaster* detects Gram-positive bacteria and fungi is activated through an endogenous ligand, namely Nerve Growth Factor-related cytokine Spaetzle (SPZ) which is processed by Spaetzle-processing enzyme (SPE). Toll receptor activation results in the recruitment of the adaptor proteins namely myeloid differentiation primary response 88 (dMyD88), Tube, and Pelle, which promotes signaling to Cactus and its ankyrin-repeat domains. Cactus is bound to the nuclear factor-kappa B (NF- κ B) transcription factors dorsal-related immunity factor (DIF) and Dorsal and following activation of the pathway, it is phosphorylated and degraded. The above signaling events result in the nuclear translocation of DIF or Dorsal that stimulate the transcriptional upregulation of antimicrobial peptide (AMP) genes, such as Drosomycin. **(B)** TLR4 receptor in *M. musculus* detects lipopolysaccharides (LPS) from Gram-negative bacteria. Myeloid differentiation primary response 88 (MyD88) is recruited with Interleukin-1 receptor-associated kinases 1 and 4 (IRAK1, IRAK4), receptor-interacting protein 1 (RIP1), and tumor necrosis factor (TNF) receptor-associated factor 6 (TRAF6). TRAF6 is self-ubiquitinated in order to recruit transforming growth factor beta (TGF- β) activated kinase 1 (TAK1) and TAK1-associated binding proteins 1 and 2 (TAB1 and TAB2). The latter leads to the activation of the I κ B kinase (IKK) complex that in turns phosphorylates the inhibitor of NF- κ B (I κ B). This leads to the release of NF- κ B that translocates to the nucleus and initiates the transcriptional induction of inflammatory and immune response related genes. This leads to the translocation of the NF- κ B transcription factors p50 and p65 to the nucleus, which in turns initiates the transcriptional induction of inflammatory and immune response related genes.

Invertebrate Models

A genetic screen for dominant suppressors and enhancers in a *Drosophila* model of A β -driven neurodegeneration revealed that Toll gene (receptor of *Drosophila* toll pathway) and key downstream components (*dif*, *pelle*, *cactus*), play a central role in mediating the neuropathological activities (34). Conversely, genetic overexpression results in accelerated deterioration of the phenotype suggesting that NF- κ B significantly enhances the pathological potential of A β (35).

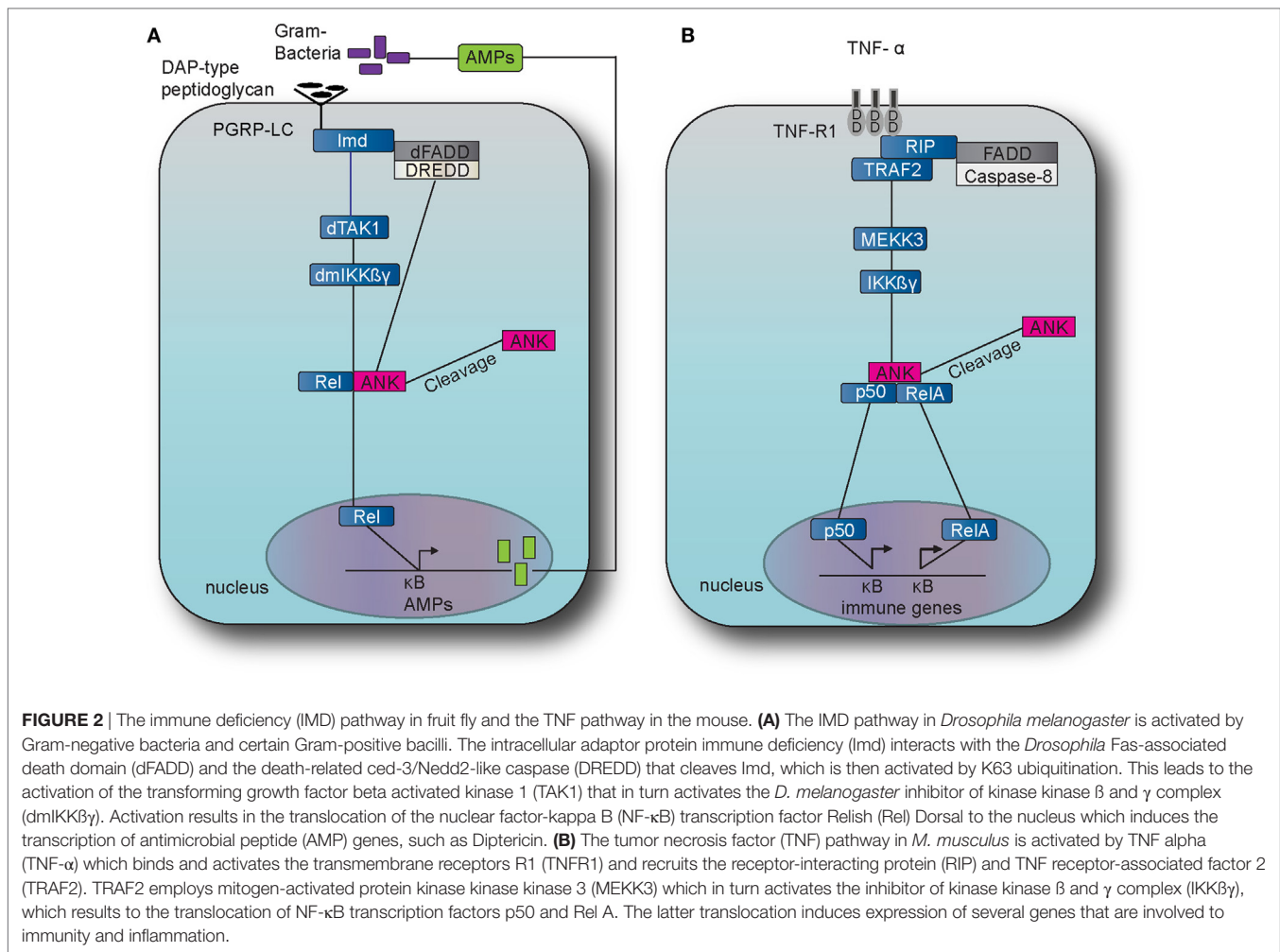
Genetic suppression of the immune deficiency (IMD) NF- κ B pathway in glial cells in a *Drosophila* model of early onset neurodegeneration dramatically rescues brain pathology, reduced activity, and short lifespan (13). Genetic overexpression of NF- κ B pathways at neuronal or glial tissue level leads to phenotypes resembling AD models with locomotor disability, accelerated neurodegeneration, and premature mortality (13).

Vertebrate Models

Age is the biggest risk factor for dementia and systemic inflammation increases as animal's age, a process known as

inflammaging (36). Microglia priming in mice induces a highly conserved transcriptional signature with aging characterized by NF- κ B expression and neuronal death (37). In rats, NF- κ B expression increases in normal aging leading to production of neurodegenerative pro-inflammatory enzymes COX-2 and iNOS (38). These changes are reversed by suppression of brain NF- κ B activation using the anti-inflammatory *Lactobacillus pentosus* var. *plantarum* C29, restoring brain-derived neurotrophic factor (BDNF) levels and memory (39). In mice, the observed positive correlation between NF- κ B activity and neuronal apoptosis suggests a role of NF- κ B in hippocampal neuroapoptosis (40). Supporting this, NF- κ B induces pro-apoptotic increases in TNF and iNOS in the hippocampus of rats exposed to neurotoxin (41).

Inactivating specific Sirtuin anti-aging genes in mice results in chronic NF- κ B overexpression leading to accelerated aging and dramatically reduced lifespan (23). In Sirtuin replete models, overexpression *via* biofeedback dysregulation results in premature aging through chronic production of excessive ROS, leading to telomere dysfunction, cellular senescence, and premature death (42). Age-related NF- κ B activation feeds into a positive



feedback loop in microglial cells causing perpetual inflammation and multiple brain responses including epigenetic suppression of GnRH genes in the hypothalamus (14). Microglial-derived NF- κ B-TNF- α axis plays a key role in homeostatic synaptic scaling, a form of synaptic plasticity. However, overexpression results in disrupted neuronal networks and behavior mimicking obsessive-compulsive disorder (OCD) (43, 44). Suppressing this pathway mediates some of the OCD-like behavioral problems in mouse models of frontotemporal dementia (44). Specifically, under-expression of NF- κ B in the mouse brain results in delayed onset of age-related pathology across all organ systems *via* preservation of the hypothalamic-pituitary-adrenal axis and GnRH levels (14).

ENVIRONMENT

Neuronal Cell Lines/Human Autopsy Studies

Type 2 diabetes mellitus (T2DM), a metabolic condition characterized by a decrease in sensitivity to endogenous insulin, is the best established environmental risk factor for the development

of AD, increasing relative risk by 50% (45). Diabetes induces A β pathology *via* NF- κ B upregulation and independent overexpression of BACE1 (46, 47). Inflammatory mediators are known to contribute to insulin resistance creating a pro-inflammatory feedback loop in diabetes (48). Administering advanced glycation end products that mimic diabetic driven pathology results in elevated BACE1 and consequent NF- κ B overexpression in both rat brains *in vivo* and neuroblastoma cells lines (49). NF- κ B suppression using Adiponectin rescues A β pathology in human T2DM neuroblastoma cells (50). Similarly, leukotriene D4, an inflammatory signaling molecule elevated in metabolic disorders, induces A β synthesis in primary neurons at 24 h with increases in NF- κ B seen after just 1 h (51, 52). Treatment of the culture with NF- κ B inhibitor pyrrolidine dithiocarbamate (PDTC) inhibited A β generation with down regulation of A β generating beta- and gamma-secretase activity suggesting NF- κ B regulates A β synthesis in metabolic disease (52).

In human neuroblastoma cells, the metabolic enzyme protein arginine methyltransferase 5 (PRMT5) regulates cellular metabolism, protecting the cell in times of stress. A β downregulates this process leading to NF- κ B overexpression, metabolic dysfunction, and premature cell death (53). Inhibiting NF- κ B reduces

apoptosis and A β deposits even in a metabolically dysfunctional organism or human cell lines suggesting a gatekeeper role for NF- κ B in maintaining metabolic homeostasis and subsequent neuroprotection independent of PRMT5 activity (53).

Invertebrate Models

In *Drosophila* models of chronic diseases including AD, the innate immune system has been identified as a key mediator of neurodegeneration (13, 34, 54, 55). NF- κ B overexpression in the fat body cells, analogous to the metabolic syndrome in humans, results in more severe neurodegeneration (56).

Supporting the role of NF- κ B in connecting whole-body metabolism with brain health, NF- κ B overexpression in the hypothalamus-like pars intercerebralis neurons in *Drosophila* results in overnutrition, impaired metabolic learning, poor memory consolidation, and metabolic disorder characterized by increased lipid levels and shortened lifespan (57). Conversely, genetic knockdown of NF- κ B signaling in glial cells leads to elevated adipokinetic and glucagon-like hormone levels, reduced glucose and lipid levels, and extension of “healthspan” (13).

Vertebrate Models

In rodent and primate models, diabetes and obesity drive overexpression of NF- κ B in the hypothalamus creating a destructive feedback loop where further NF- κ B expression promotes hypertension, overnutrition, and decreased insulin sensitivity (58–61). Injecting A β into the brains of mice and macaques results in an increase in NF- κ B in the cell nuclei of the hypothalamus and subsequent induction of peripheral glucose intolerance (62). In this model, pharmacological inhibition of NF- κ B maintained peripheral metabolic homeostasis. Inducing diabetes in rats results in hippocampal NF- κ B-dependent neurodegeneration *via* disruption of CREB phosphorylation, reducing levels of protective downstream proteins including BDNF (63).

The tetracycline derivative Minocycline inhibits NF- κ B and prevents further A β deposition in a mouse model of diabetes-driven AD. BACE1 activity remained elevated demonstrating an NF- κ B-dependent protective mechanism (29). Mice fed on high-fat diets demonstrate elevated brain BACE1 expression as do transgenic diabetic mice. Administration of the anti-inflammatory agent all-trans-retinoic acid reduces BACE1 expression in both WT and mutant but this effect is abolished when the NF- κ B-binding site at the promoter region of BACE1 is mutated (64).

EMERGING RISK FACTORS

Alcohol Intake

In *Drosophila*, alcohol consumption activates Toll-NF- κ B signaling increasing ethanol resistance and gene products known to be outputs of innate immune signaling are rapidly induced following ethanol exposure (65). Ethanol treatment of cultured hippocampal rat neurons causes a dose- and time-dependent increase in NF- κ B-DNA-binding activity, resulting in significant upregulation of inflammatory markers and increased susceptibility to neurotoxins; reversible by applying NF- κ B inhibitors (66, 67).

Opposing, the consumption of moderate amounts of alcohol, particularly red wine, is associated with a reduced risk of AD. Anthocyanin, a polyphenol found in wines, protects rat hippocampal neurons against oxidative stress *via* NF- κ B suppression (41, 68).

Sleep

Sleep quality and well-being are symbiotic and reduced sleep quality is increasingly being associated with increased risk of dementia. Sleep-wake cycle homeostasis is important in the processing and removal of A β plaques which, in turn, are known to dysregulate this reparative process (69). Sleep disruption and deprivation are known to cause over expression of the NF- κ B pathway in hippocampal cell cultures, fruit flies, rodents, and humans (70–73). Improvement in sleep quality in older adults is correlated with a reduction in circulating NF- κ B (74).

Traumatic Brain Injury (TBI)

Traumatic brain injury activates both microglia and astrocytes and induces self-sustaining inflammatory responses in the brain *via* NF- κ B activation (75, 76). In *Drosophila*, flies with TBI exhibited temporary incapacitation, ataxia, activation of the innate immune response, neurodegeneration, and death similar to humans with TBI (77, 78). Rat models have demonstrated the acute onset and prolonged overexpression of NF- κ B in brain regions most commonly associated with post-injury atrophy (79, 80). Recent studies have shown NF- κ B to be significantly elevated in the ipsi-lateral cortex of both adult and old TBI mice in a time-dependent manner (81). These interactions started immediately post-injury in the old mice compared to the adult mice suggesting an age-related failure of NF- κ B suppression.

PROTECTIVE FACTORS

Exercise

Under expressing NF- κ B in mice results in greater endurance, cognitive performance, and resistance to obesity (82). An aerobic exercise protocol in wild-type rats attenuated age-related memory decline and decreased hippocampal NF- κ B levels and atrophy (83). In rats fed a pro-inflammatory diet and subjected to either strength training, aerobic exercise or a combination of both, all protocols reduced liver and muscle NF- κ B levels to pre-diet levels (84). These findings are supported by studies demonstrating the indirect suppression of NF- κ B *via* cytokine IL-10, a potent NF- κ B inhibitor, induced by exercise (85).

Diet

Curcumin, a constituent of turmeric, has gained much attention in recent years for its potential as a neuroprotective compound. In a *Drosophila* model of neurodegeneration, the curcumin analog C150 significantly reduced neuronal cell death, increased healthy lifespan, and reduced DNA mutation in brain tissue by suppressing NF- κ B (86).

The neuroprotective effects resulting from the Mediterranean diet or those rich in oily fish may be mediated *via* the anti-inflammatory properties of key nutrients (87). A β induced increases in the translocation NF- κ B subunits is attenuated in the presence of

tyrosol (Tyr) and hydroxytyrosol (OH-Tyr) found abundantly in olive oil (88). Transgenic increases in omega-3 polyunsaturated fatty acid in the brain of mice reduces the inflammatory response to LPS challenge *via* NF- κ B pathways (89).

The omega 3 fatty acid eicosapentaenoic acid indirectly downregulates NF- κ B expression acting as a ligand at peroxisome proliferator-activated receptor gamma, a regulator of fatty acid storage and glucose metabolism, and reduces symptoms of depression (87, 90). In AD-mouse hippocampal slices, food-derived anti-oxidants provide neuroprotection and reduce A β *via* the anti-inflammatory properties of the polyphenolic compounds within them (91). Specifically, the phenolic compound resveratrol reduces A β -induced microglial activity and neuroinflammation *via* NF- κ B suppression in murine microglial and macrophage cells (92).

Anti-inflammatory Drugs

The prolonged use of non-steroidal anti-inflammatory drugs (NSAIDs) is associated with a reduction in the AD risk (93). In primary rat, neurons and human neuronal cell lines NSAIDs strongly inhibit NF- κ B-driven expression of BACE1 activity preventing the cleavage of A β from APP (28, 94). Long-term administration of potent NSAID indomethacin blocks activation of NF- κ B and significantly reduced the amyloid pathology in transgenic AD mice (95).

Aspirin, an NSAID derived from salicylic acid, completely inhibits A β activation of the NF- κ B pathway, reducing levels of pro-inflammatory cytokines and chemokines, and increasing levels of anti-inflammatory IL-10 in rodent microglia and neurons resulting in recruitment of A β phagocytic microglia and improved cognitive and synaptic functioning (28, 96).

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CONCLUSION

Epidemiological studies are beginning to converge of common risk factors for the development of AD with strong signals also emerging for certain protective factors. The emergence of NF- κ B as a regulator of aging and proliferation of studies implicating NF- κ B over-activation in a number of neurodegenerative diseases suggests that it may be important in modulating the risk of disease. This review has highlighted the intimate relationship between all known and emerging risk and protective factors for Alzheimer's and NF- κ B activity, implicating over-activation with an increased risk of the disease and suppression being associated with risk reduction. Future work, both in models of disease and trials in man, should focus on therapies that directly target NF- κ B overexpression to explore whether early risk identification and targeted anti-inflammatory treatment can significantly increase the time of disease onset and, consequently, reduce the incidence of this devastating disease.

AUTHOR CONTRIBUTIONS

SJ and IK wrote the manuscript, and IK designed the figures.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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