

Nuclear factor-kappa B signaling pathways and parasitic infections: An overview

Review
Article

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ABSTRACT

Host nuclear factor-kappa B (NF- κ B) transcription factor plays a pivotal role in innate immunity and resistance to infection. It induces the expression of several genes that encode pro-inflammatory cytokines. It also participates in regulating the differentiation and survival of innate immune cells and lymphocytes. Infection of host cells with pathogens usually activates host NF- κ B signaling pathways. The majority of parasites evolved diverse protective mechanisms against NF- κ B activity to shield their continued existence. Herein, we present brief insights into NF- κ B signaling pathways, activators and inhibitors, and the main subsequent events following protozoan and helminthic infections *in vitro*, as well as *in vivo* either in experimental models, or in humans. Understanding the underlying mechanisms of NF- κ B activation and inhibition may be of great help as a therapeutic strategy against different parasitic infections.

Keywords: helminths; innate immunity; novel drugs; nuclear factor-kappa B; pro-inflammatory cytokines; protozoa; toll-like receptors.

Received: 18 August, 2021, **Accepted:** 10 October, 2021.

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Print ISSN: 1687-7942, **Online ISSN:** 2090-2646, **Vol. 14, No. 3, December, 2021.**

Abbreviations: DCs: Dendritic cells; ECs: Endothelial cells; ESPs: Excretory-secretory products; IKK: Inhibitor of nuclear factor-kappa B kinase; IKK α : Inhibitor of NF- κ B kinase- α ; IKK β : Inhibitor of NF- κ B kinase- β ; IL: Interleukin; iNOS: Inducible nitric oxide synthase; I κ B: Inhibitor of NF- κ B; I κ B α : Inhibitor of NF- κ B- α ; LPS: Lipopolysaccharide; NF- κ B: Nuclear factor-kappa B; NO: nitric oxide; TLR: Toll-like receptor; TNF- α : Tumor necrosis factor- α .

INTRODUCTION

Host responses to infection are generally categorized into innate and adaptive immune responses. Foreign pathogens stimulate the host immune response with associated host and pathogen cascades. Consequently, signal transduction events induce changes in gene expression, followed by release of cytokines and other factors such as inflammatory mediators and chemokines that mediate recognition^[1].

Induced transcriptional changes are controlled by NF- κ B signaling. Notably, NF- κ B was first identified in active B cells by its binding affinity to immunoglobulin κ light-chain gene transcriptional enhancer^[2]. Besides, NF- κ B serves as dominant modulator of immunoinflammatory responses. Members of NF- κ B are usually found in the cytoplasm in a dormant state. Following stimulation of the innate immune receptors, NF- κ B becomes activated for nuclear translocation and activation of gene transcription^[3]. Its signaling activity is essential for utmost transcription of many inflammatory mediators, chemokines, and cytokines in immune cells. Suppressing the activity of NF- κ B signaling can be advantageous in controlling several disease inflammatory processes, including parasitic infections that manipulate NF- κ B in different ways. However, constant NF- κ B inhibition can be associated with intense adverse effects. Therefore, interpretation of the full dynamics of the pathological

consequences of NF- κ B activation in different diseases is fundamental^[4,5].

Signaling pathways

Family members of NF- κ B include inducible transcription factors that control an enormous array of genes concerned with inflammation and immunity^[6]. There are five structurally related members; NF- κ B1 (p50), NF- κ B2 (p52), RelA (p65), RelB, and c-Rel, that control target genes transcription by binding to κ B enhancer (a peculiar DNA element), as homo- or hetero-dimers^[7]. Normally, inhibitory proteins are responsible for cytoplasmic sequestration of NF- κ B proteins. These include inhibitor of nuclear factor-kappa B (I κ B) family members in addition to related proteins, such as inhibitor of NF- κ B- α (I κ B α) as the most significant inhibitor. It is worth mentioning that I κ B α degradation is induced by inhibitor of nuclear factor-kappa B kinase (IKK) complex through site-specific phosphorylation. It is observed that IKK contains two catalytic subunits: inhibitor of NF- κ B kinase- α (IKK α) and inhibitor of NF- κ B kinase- β (IKK β); besides inhibitor of NF- κ B kinase- γ (IKK γ), which is a regulatory subunit known as NF- κ B essential modulator (NEMO)^[8].

Two main pathways activate NF- κ B signaling; the classical (canonical) and alternative (non-canonical or atypical) pathways (Figure 1). The first is associated

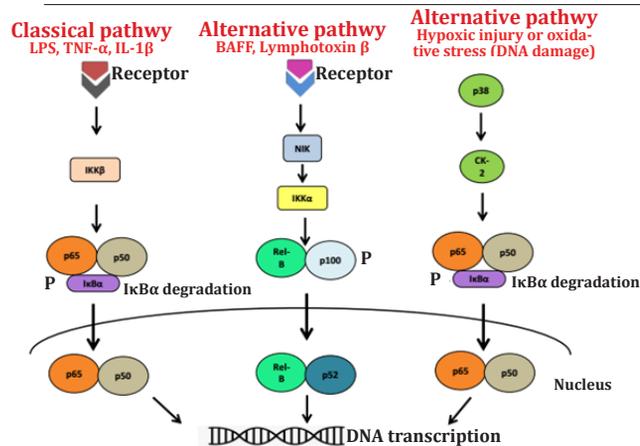


Fig. 1. Nuclear factor-kappa B signaling pathways. Activators of NF- κ B bind corresponding receptors and activate IKK β in the classical pathway and IKK α via NIK in alternative pathway. DNA damage acts through p38 and CK-2. Kinases phosphorylate I κ B α , and p100. Active dimers of NF- κ B are released, translocate to the nucleus, and bind DNA to start target genes transcription. Illustrated by Alhusseiny SM.

BAFF: B-cell activating factor; **CK-2:** Casein kinase-2; **I κ B α :** Inhibitor of NF- κ B- α ; **IKK α :** Inhibitor of NF- κ B kinase- α ; **IKK β :** Inhibitor of NF- κ B kinase- β ; **IL-1 β :** Interleukin-1 β ; **LPS:** Lipopolysaccharide; **NIK:** NF- κ B inducing kinase; **P:** Phosphorylation; **TNF- α :** Tumour necrosis factor- α .

with immune and inflammatory responses, whereas the latter is a secondary signaling pathway concerned with controlling adaptive immunity, B cell function, and lymphoid organ development^[1]. Activators of the classical pathway include lipopolysaccharide (LPS), tumor necrosis factor- α (TNF- α), or interleukin (IL)-1 β , that attach to specific receptors. Sequences include IKK β -mediated phosphorylation of I κ B α that becomes degraded through the proteasome releasing p50/p65 dimers. On the other hand, the alternative pathway is induced by B-cell activating factor (BAFF) or lymphotoxin β . It includes IKK α activation through NF- κ B inducing kinase (NIK), p100 phosphorylation by IKK α , and its processing by the proteasome to yield p52. Next steps include liberation of RelB/p52 dimers, nuclear translocation, and triggering specific genes, which are different from the genes controlled by the classical pathway. In the atypical pathway, hypoxic injury or oxidative stress (DNA damage) leads to massive I κ B α phosphorylation via p38-induced casein kinase-2 (CK-2) and release of p50/p65 dimers^[9].

Role of NF- κ B signaling in immune response and inflammatory process

Extensive studies have been conducted on NF- κ B signaling pathways owing to its major role in activating plentiful genes implicated in the infection response. Cell surface toll-like receptors (TLRs) expressed via dendritic cells (DCs), mucosal epithelial cells, and macrophages are the main component of the innate immune response that identifies pathogens (cell wall LPS and nucleic acids)^[10]. Once triggered, all TLRs endorse the NF- κ B signaling pathways^[11]. These NF- κ B pathways elicit macrophage inflammatory protein-1 α (MIP-1 α), pro-inflammatory cytokines (e.g. TNF- α , IL-1, and IL-6), besides adhesion molecules such as vascular cell adhesion molecule-1 and E-selectin expression^[10]. It was reported that E-selectin belongs to cell adhesion molecules that are expressed by cytokines-activated endothelial cells (ECs). It is designated as CD62E, and is recognized to have chief effects during the inflammation response^[12]. After release, TNF- α and

IL-1 reactivate NF- κ B pathway, with further response augmentation. Moreover, NF- κ B activates B and T lymphocytes receptors (co-stimulatory and antigen), besides supporting the survival and differentiation of B cells through B-cell activating factor activity^[13].

Inhibition of NF- κ B signaling pathways

In most cells, activation of NF- κ B pathway is usually a temporary process (~30-60 min). Persistent NF- κ B signaling is inhibited by down-regulation of NF- κ B within a feedback loop (negative feedback). The gene that encodes I κ B α is provoked by NF- κ B, and newly-formed I κ B α enters the nucleus, eliminates NF- κ B from DNA, and sends the complex again to the cytoplasm. There, NF- κ B is retained till further activation^[13]. Dysregulation of NF- κ B is associated with diverse health problems, e.g. multiple sclerosis, cerebral stroke, Alzheimer's disease, atherosclerosis, bronchial asthma, diabetes mellitus, viral infections, and genetic disorders. Proper control of NF- κ B signaling through pharmacological or genetic therapies represents a novel strategy for the management of NF- κ B related disorders^[14].

Interestingly, NF- κ B signaling pathways can be prevented through many pharmacological agents that inhibit NF- κ B activity at different levels including:

- **Non-steroidal anti-inflammatory drugs** (e.g. sodium salicylate and aspirin) act by inhibiting cytokine and endotoxin-induced nuclear translocation of NF- κ B through preventing phosphorylation and proteolysis of I κ B α ^[15]. Besides, they can serve as IKK inhibitors and act by specifically inhibiting ATP-binding to IKK β . Consequently, they markedly decrease IKK β -dependent phosphorylation, and prevent both IKK β proteasomal degradation and NF- κ B pathway activation^[16].
- **Steroids** (e.g. glucocorticoids) that directly interact with NF- κ B and activator protein-1 (AP-1), and produce independent transrepression complexes. They can also provoke the expression of I κ B α and hold NF- κ B in the cytoplasm^[17].

- **Anti-oxidants** derived from natural agents (e.g. green tea) that inhibit I κ B α degradation provoked by TNF- α , and activation of NF- κ B^[18].
- **Proteasome inhibitors** (e.g. peptidyl aldehydes and lactacystin) that can effectively block ubiquitin-dependent protein degradation through their ability to inhibit I κ B α degradation^[19].
- **Casein kinase-2 inhibitors** (e.g. the natural flavone apigenin found in tea, onions, and oranges) that act by stabilizing I κ B α and down-regulating NF- κ B activity^[20].

Signaling pathways of NF- κ B in parasites

It is well documented that NF- κ B proteins are nearly expressed by all cells, and are implicated in lymphocytes development, maturation, proliferation as well as survival. Moreover, the expression of variable genes types is coordinated by NF- κ B. These genes have crucial roles in host innate and adaptive immune responses, and are strongly associated with inhibition of apoptosis. Interestingly, parasites attack various NF- κ B pathway steps, leading to interference with the activity of genes responsible for the immune response. The biology of the different parasites is pivotal in controlling the patterns and kinetics of NF- κ B activity and hence the subsequent immune responses^[4].

Protozoa

Entamoeba histolytica

In RAW 264.7 cells culture, Gal-lectin caused time- and concentration-dependent elevated expression of mRNA levels of TLR2, and induced NF- κ B p50 and p65 nuclear translocation. The use of SN50 (a specific cell-permeable NF- κ B inhibitory peptide), pyrrolidine dithiocarbamate (PDTC), and SB 208530 (to inhibit p38 activity) reduced the expression of TLR-2. Inhibition of mitogen-activated protein kinase (MAPK) activity through SB203580 (p38 kinase specific inhibitor) caused significant reduction of Gal-lectin-induced TLR-2 mRNA induction^[21]. These findings point to the potential importance of targeting TLR to limit pathological changes in the intestine in response to entamoebiasis *histolytica*^[21].

In addition, the effects of *E. histolytica* Gal-lectin on DCs in mice was investigated in 2007 by Ivory and Chadee^[22], who documented that Gal-lectin induced DCs maturation, and up-regulated MHC class II and maturation markers (CD40, CD80, and CD86), besides increasing mRNA levels of IL-12. They also reported that Gal-lectin caused NF- κ B p65 nuclear translocation, as well as extracellular signal regulated protein kinase 1 and 2 (ERK 1/2), JNK, and p38 phosphorylation. In spite of stimulating NF- κ B p65 activity, the valuable effects of Gal-lectin on DCs maturation could be exploited as a lead molecule for vaccine development^[22].

In 2010, Cho *et al.*^[23] investigated NF- κ B p50 subunit activity in murine amoebiasis and reported

that its selective deletion in mice increased their susceptibility to infection. This finding reflects that NF- κ B activity, especially activity of the p50 subunit, is partially responsible for rapid clearance and resistance to *E. histolytica* infection. The use of calpeptin (calpain inhibitor) inhibited degradation of *Entamoeba*-induced NF- κ B, signal transducer and activator of transcription 3 and 5 (STAT3 and STAT5). Degradation of NF- κ B by cysteine protease was found to hasten cell death^[24].

In human monocytes, NF- κ B pathway operates through monocyte locomotion inhibitory factor (MLIF). This oligopeptide is heat-stable, detected in *Entamoeba* axenic cultures, and was documented to contribute to late inflammation in amoebic liver abscess by inhibiting amoebic motility. Furthermore, it prevents the synthesis of adapter protein myeloid differentiation factor 88 (MyD88), increases p50/p50 homo-dimer translocation, and alters translocation and dynamics of p65/p50 heterodimer. In addition, this factor possesses specific anti-inflammatory activities in both *in vivo* and *in vitro* models. It inhibits respiratory burst and human monocyte locomotion in neutrophils and monocytes, inhibits vascular cell adhesion molecule-1 and very late antigen-4 (VLA-4) expression, and increases anti-inflammatory cytokines expression^[25,26]. Activity of MLIF manifests clinically as less invasive intestinal amoebiasis (produced by less virulent strains)^[27], and it is not secreted by commensal *E. dispar*^[28]. Protective effects of MLIF have been demonstrated in rats, where it reduced cellular inflammatory infiltrate and significantly reduced inflammatory cytokines levels in contused tibia^[29]. It also protects mice against cerebral malaria^[30]. Based on most represented studies, it is worth mentioning that the gut epithelial cells exhibit a unique feature in *E. histolytica* infection, where NF- κ B activity is associated with a healing response, not a pro-inflammatory one^[31].

Giardia lamblia

In 2012, Lee *et al.*^[32] evaluated the signaling sequences following *in vitro* incubation of HT-29 gastrointestinal cell line with *G. lamblia* trophozoites; WB and GS strains that belong to assemblages A and B, respectively^[33]. They demonstrated increased levels of TNF- α , IL-1 β , and IL-8 (with higher levels in GS-infected cells). The researchers^[32] also proved that *G. lamblia* excretory-secretory products (ESPs) induced IL-8 production by provoking signaling activities of mitogen-activated protein kinases; p38 and extracellular signal regulated protein kinase 1 and 2, besides activator protein-1, and NF- κ B. They also reported reduced IL-8 levels in response to the use of SB202190 (a p38 specific inhibitor) or inhibiting ERK1/2 activity by PD98059^[32].

Giardia lamblia significantly inhibited inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) expression, and nitric oxide (NO) production

in *in vitro* cultured LPS-stimulated RAW 264.7 cells or human monocyte-derived macrophages. These mechanisms help *Giardia* evade the host immune response. Moreover, *G. lamblia* cleaved the p65 subunit of NF- κ B in LPS-stimulated cells, and suppressed IL-1 β , IL-6, and IL-10 expression. These effects were attributed to *Giardia* proteases; two cysteine proteases and a metalloprotease^[34].

Cryptosporidium parvum

In biliary epithelial cell cultures formed of human bile duct epithelial cells transformed by simian virus 40 (SV40), *C. parvum* triggered NF- κ B signaling, and increased IL-8 level. Infection also caused NF- κ B p50 and p65 nuclear translocation and I κ B α degradation. In addition, marked apoptosis was observed in infected cells following inhibition of NF- κ B activity through MG-132 (a peptide-aldehyde proteasome inhibitor) or SN50 (a specific cell-permeable NF- κ B inhibitory peptide). This finding denotes that anti-apoptotic effects of *Cryptosporidium* are related to triggering NF- κ B^[35].

Since *C. parvum* resides at the apical border of enterocytes with little invasion, epithelial cells innate immunity is crucial for protection of the host. Both NF- κ B activity and TLR play important roles in innate immune response against *Cryptosporidium*^[36].

In 2011, O'Hara *et al.*^[37] investigated the role of TLRs4 in *C. parvum* infection in mice, and documented significant reduction in *Cryptosporidium* oocysts four weeks post-infection (wpi), and absence of excretion of oocysts in stool three wpi in wild type mice, compared to TLR4-deficient mice. They also reported decline in hepatic IL-6 and interferon- γ (IFN γ) levels at one and two wpi, respectively, in wild type mice. A different pattern was detected in TLR4-deficient mice, where IL-6 and IFN- γ levels remained high. In addition, they documented reduction in serum liver enzymes (alanine transaminase, aspartate transaminase, and alkaline phosphatase) levels in wild type mice, and persistently high levels in TLR4-deficient mice. They also verified the presence of normal histological structure four wpi in wild type mice, whereas TLR4-deficient mice showed severe inflammation in the portal tracts, severe hepatocellular necrosis, and severe proliferation in cholangiocytes. These findings indicate that TLR4 activity can effectively eradicate biliary cryptosporidiosis^[37].

Leishmania spp.

In 2004, Guizani-Tabbane *et al.*^[38] studied the effects of stimulating NF- κ B activation in both fresh human monocytes and promonocytic human cell line U937 infected with *L. major*. The authors proved that provocation of nuclear translocation of NF- κ B p65/p50 complex following phorbol-12 myristate-13 acetate (PMA)-therapy was inhibited by *Leishmania* in the PMA-treated differentiated U937 cells. They also documented that the expression of NF- κ B complexes (containing p50-

and c-Rel) was activated in fresh human monocytes and U937 cells. In addition, reduced *Leishmania*-provoked IL-10 and TNF- α - production in differentiated U937 cells, in response to caffeic acid phenethyl ester (CAPE) used to suppress NF- κ B activity was demonstrated^[38].

Cutaneous leishmaniasis is associated with a powerful healing response as a result of a robust Th1 immune response in vertebrates. In *L. major*-infected human DCs, IL-12 was found to be up-regulated, compared to *L. donovani* which does not provoke IL-12 production; because induction of IL-12 production differs based on *Leishmania* species and strain^[39]. In 2008, Jayakumar *et al.*^[40] studied the molecular events underlying IL-12 induction in human DCs infected with *L. major* NIH Friedlin V1 (MHOM/IL/80/FN) strain and *L. donovani* strain 9515 (MHOM/IN/95/9515). The authors reported significant up-regulation of the levels of IL-12 p35 and IL-12 p40, as well as progressive increase in interferon regulatory factor-1 (IRF-1) and factor-8 (IRF-8) mRNA levels in DCs infected with *L. major*, compared to DCs infected with *L. donovani*. Moreover, they detected increased nuclear translocation of NF- κ B p65, NF- κ B p50, c-Rel, IRF-1, and IRF-8, following *L. major* infection, in comparison with cells infected with *L. donovani*. Jayakumar *et al.*^[40] also documented up-regulation of guanylate binding proteins-1 and -2 (GBP-1 and GBP-2), as well as cyclooxygenase-2 genes. These genes were observed to be up-regulated in response to *L. major* infection^[41,42]. These findings are associated with up-regulation of IL-12 levels that will manifest as a powerful healing response. In addition, Jayakumar *et al.*^[40] reported that inhibition of NF- κ B activation by pre-treatment of DCs using caffeic acid phenethyl ester significantly reduced the mRNA levels of IL-12 p35, IL-12 p40, IRF-1, and IRF-8 in treated DCs infected with *L. major*, compared to infected untreated cells.

Regarding *L. amazonensis*, infection proved to be associated with activating NF- κ B p50/p50 complex, stimulating the conversion of NF- κ B p65/p50 to NF- κ B p50/p50 complex in LPS-activated macrophages, and decreasing p65 levels in infected macrophages. Activation of the NF- κ B repressor complex p50/p50 decreases mRNA levels of the iNOS, and NO production in LPS-stimulated macrophages. These effects support the parasite to evade immune attack by the host^[43].

Trypanosoma cruzi

The course of *in vitro* *T. cruzi* trypomastigotes (Tulahuen strain) infection in human umbilical vein endothelial cells (HUVEC) culture was studied by Huang *et al.*^[44]. They demonstrated that infected TNF- α -treated HUVEC showed up-regulation of intercellular adhesion molecule-1 (ICAM-1), significant induction of vascular cell adhesion molecule-1 and E-selectin mRNA expression, with persistent elevation of expression of these adhesion molecules in infected cells. They also

documented NF- κ B activation (p50 and p65) in *T. cruzi*-infected HUVEC. According to the researchers, these findings indicate that initiation of NF- κ B activity in endothelial cells following *T. cruzi* infection may contribute to the pathology of chronic Chagas cardiomyopathy, caused by inflammation, subsequent vascular injury, and dysfunction of endothelium.

In 2000, Hall *et al.*^[45] investigated NF- κ B signaling in *T. cruzi* (Silvio, MV13, and Tulahuén strains) cultivated in mink lung epithelial cells (Mv1lu), and demonstrated that trypomastigotes induced DNA binding and nuclear translocation of p65 subunit, and NF- κ B-dependent gene expression. In addition, trypomastigotes activated nuclear translocation of NF- κ B of human fibroblasts and murine endothelial cells SVEC4-10 *in vitro*, but did not activate NF- κ B or stimulate p65 translocation in muscle-derived cells (primary cultures of bovine or human smooth muscle cells, rat cardiac myoblast line H9C2, or rat skeletal muscle myoblast line L6E9)^[45].

The signaling events underlying *T. cruzi* (Y strain)-induced cardiomyocyte hypertrophy were studied by Petersen *et al.*^[46] who documented that NF- κ B is implicated in cardiomyocyte hypertrophy and IL-1 β production provoked by *T. cruzi*. They reported that *T. cruzi* trypomastigotes significantly increased the mean cell size in rat neonatal cardiomyocytes, and that pre-treatment of cardiomyocytes with SN50 prevented hypertrophy in *T. cruzi*-infected cells; implying that NF- κ B signaling is needed for *T. cruzi*-induced cardiomyocyte hypertrophy. They also demonstrated completely inhibited hypertrophy in cardiomyocytes pre-treated with TLR2 antibodies; while antibodies to TLR4- did not inhibit *T. cruzi*-induced hypertrophy. Besides, they documented increased IL-1 β production in *T. cruzi*-infected cardiomyocytes and its reduction in response to TLR2-blocking antibody, increased activation of NF- κ B reporter in cells infected with *T. cruzi*, and reduced reporter activation in cardiomyocyte cultures pre-treated with TLR2-blocking antibody^[46].

In Chagas' disease, cardiomyocyte apoptosis is a critical determinant of the course of the disease. The host apoptotic pathways in *T. cruzi* (Y strain) infection differ according to the cell type, i.e., *T. cruzi*-infected cardiomyocytes and neuronal cells were protected from apoptosis, whereas T cells showed activated apoptotic response in cardiomyocytes infected with *T. cruzi*. *In vitro* protection of cardiomyocytes against apoptosis was elicited by TNF- α . The decrease in TNF- α serum levels was correlated to both parasite burden and stimulation of NF- κ B activity. In addition, inhibition of apoptosis diminished activation of caspase-3 (central mediator of apoptosis). Inhibition of apoptosis in *T. cruzi*-infected cardiomyocytes is probably a key event in maintaining infection of the cardiac muscles with diminishing tissue injury, and in apoptosis-concomitant cardiomyocyte reorganization^[47].

In 2011, Pinto *et al.*^[48] archived that TNF treatment significantly increased the susceptibility of two epithelial cell lines (LLC-MK2 cells and HEK293T) to infection by *T. cruzi* (Y strain) in a concentration-dependent manner. It also increased cells susceptibility to trypanosomiasis after exposure to supernatants from *T. cruzi*-infected human monocytes (phorbol myristate acetate-differentiated). The use of monoclonal antibody to TNF and TPCK (a NF- κ B inhibitor) significantly reduced parasite burden.

Trichomonas vaginalis

Incubation of *T. vaginalis* (lysates, opsonized-live, and live) with human monocyte-derived macrophages (HMDM) increased NF- κ B p65 nuclear translocation and phosphorylation, and increased the levels of IL-1 β , IL-6, and TNF- α . Furthermore, live *T. vaginalis* markedly increased generation of NO and expression of iNOS levels in HMDM. The use of the NO synthase inhibitor L-NMMA significantly inhibited NF- κ B activation and TNF- α production induced by trichomonads in macrophages. Pre-treatment with pyrrolidine dithiocarbamate or Bay11-7082 (NF- κ B inhibitor) decreased TNF- α produced from human macrophages. These findings indicate that TNF- α , NO, IL-1, and IL-6 are released by macrophages in response to *T. vaginalis* infection, and that TNF- α production in macrophages is dependent on NO-dependent activation of NF- κ B. These events might be implicated in inflammation caused by *T. vaginalis*^[49].

Toxoplasma gondii

Toxoplasma gondii-infected HeLa cells showed provoked NF- κ B signaling activity, and increased mRNA expression of monocyte chemotactic protein-1 (MCP-1) and IL-8. The use of calpain-1 inhibitor to inhibit NF- κ B signaling significantly decreased *Toxoplasma*-provoked MCP-1 and IL-8 secretion. This implies that NF- κ B masters chemokine activity in cells infected with *T. gondii*, and that the pathogenic effects of *T. gondii* are related to MCP-1 and IL-8 secretion, as well as the subsequent staffing of monocytes, neutrophils, and lymphocytes^[50].

Shapira *et al.*^[51] investigated the effects of toxoplasmosis both *in vivo* and *in vitro* on NF- κ B activity. Markedly increased levels of its binding complexes (mainly p50 and p65) were demonstrated in peritoneal cells from *T. gondii* RH strain-infected mice, compared to uninfected mice. In addition, a significant increase in NF- κ B (mainly p50) level was recorded denoting that *in vivo* toxoplasmosis activates NF- κ B signaling. They also demonstrated cytoplasmic localization of NF- κ B p65 and c-Rel in unstimulated bone marrow (BM)-derived macrophages, and in LPS-stimulated cells; and the absence of p65 or c-Rel signaling in *T. gondii* RH tachyzoites-infected cells. Besides, they documented activation of p65 in BM-derived macrophages exposed only to LPS, and no response in cells cultured

with tachyzoites in response to LPS, implying that infection with *Toxoplasma* can dynamically influence the sequences causing NF- κ B nuclear translocation following pro-inflammatory stimuli^[51].

The signaling activity of NF- κ B and apoptotic cascade-implicated genes expression in murine embryonic fibroblasts (MEFs; wild-type) infected with *T. gondii* (RH strain) were studied by Molestina *et al.*^[52]. They reported p65 and p50 nuclear translocation in infected MEFs after TNF stimulation, compared to uninfected cells that did not display nuclear localization of the two subunits. They also reported provoked phosphorylation of I κ B, and its recruitment on the parasitophorous vacuole membrane, signifying a direct *Toxoplasma* effect that could be related to indigenous agents modulating the host NF- κ B activity to guard parasite viability^[52]. The investigators also documented different patterns of gene expression in wild-type and p65^{-/-} MEFs following infection with *T. gondii*. Increased anti-apoptotic proteins Bcl-XL and Bfl-1 expression was demonstrated in wild-type MEFs, in comparison with p65^{-/-} MEFs. Moreover, infected wild-type MEF displayed increases in the expression of inhibitors of apoptosis-1 and -2 (IAP1 and IAP2), and neuronal apoptosis inhibitory protein-1 (NAIP-1), compared to p65^{-/-} cells. These results suggested that anti-apoptotic genes up-regulation is provoked by activated NF- κ B signaling, and can manifest clinically as enhanced survival of *Toxoplasma*^[52].

In 2005, Shapira *et al.*^[53] evaluated the impact of *Toxoplasma* tachyzoites infection on NF- κ B-induced TNF- α and IL-12 expression in human foreskin fibroblasts (HFF) and found no nuclear accumulation of p65/RelA in infected cells. They also demonstrated marked IKK activity and I κ B α degradation in *T. gondii*-infected cells, besides markedly delayed I κ B α synthesis. In addition, the authors reported that interrupting proteins nuclear transport mediated by chromosomal region maintenance-1 (CRM1) using leptomicyne B (LMB) was accompanied with NF- κ B nuclear accumulation in cells (infected and non-infected). The authors concluded that this indicates that NF- κ B dimers nucleocytoplasmic transportation in unstimulated cells is not affected by *T. gondii*.

Toxoplasma rhoptry protein 18 (ROP18) was documented to interact with p65 subunit of NF- κ B and mediate its proteasome-ubiquitin-dependent degradation that is ROP18 kinase-dependent^[54]. These authors found that RAW 264.7 cultured cells expressing ROP18-WT (wild-type ROP18 RH strain) had low TNF- α , IL-6, and IL-12 p40 levels, compared to kinase-deficient ROP18 RH strain. Also, U937 cells expressing ROP18-WT RH suppressed LPS-induced cytokine production, compared to cells infected with kinase-deficient ROP18 RH strain. These findings implied that inhibition of NF- κ B by ROP18 is associated

with reduced levels of pro-inflammatory cytokines, which favors *Toxoplasma* persistence^[54].

***Plasmodium* spp.**

Transfer of the circumsporozoite protein (CSP) from the parasitophorous vacuole to the hepatic cytoplasm was recorded as vital for sporozoites development during exo-erythrocytic cycle of *Plasmodium*, owing to its blocking effect on NF- κ B activation in hepatocytes. This protein acts by down-regulating the expression of many genes controlled by NF- κ B (inflammation-implicated genes), and by affecting the expression of host genes accountable for different metabolic processes to create a suitable milieu for parasite growth^[55].

In 2018, Deng *et al.*^[56] studied the effects of CSP as a potential NF- κ B inhibitor on the growth of the human lung cancer cell line A549, and reported suppressed proliferation and induced apoptosis of its cells, following transfection with a recombinant plasmid expressing CSP. The suppressed TNF- α -induced activation in response to both CSP and its nuclear localization signal (NLS) motif, markedly inhibited NF- κ B nuclear translocation in A549 cells stimulated with TNF- α .

The pathogenesis of cerebral malaria (CM) in *P. falciparum*-infected patients involves cytokines release and microvascular sequestration of parasitized RBCs^[57], in addition to signaling factors, such as NF- κ B^[58]. Punsawad *et al.*^[59] investigated NF- κ B p65 and cleaved caspase-3 expression and their association with apoptosis in brain tissues specimens from patients with CM and non-cerebral malaria (NCM), and normal brains (deceased persons in accidents with no head insult). They recorded positive NF- κ B p65 nuclear staining (activated NF- κ B) in glial cells of the white matter, and in areas with brain injury (Durck's granuloma; reactive gliosis that closely looks like granulomas, and areas of ring haemorrhage). The presence of significant positive correlations between neuronal NF- κ B p65-expression and the total score for histopathological changes was also documented. This indicated that the histopathological changes in CM are moderated by signaling through p65 subunit of NF- κ B^[59]. In addition, they detected an intense NF- κ B p65 nuclear immunoreactivity in vascular ECs, as well as in circulating inflammatory cells (especially lymphocytes) in cases with CM. Besides, the higher nuclear NF- κ B p65 immunoreactivity in intravascular lymphocytes, compared to NCM and controls denoted an active state during malaria infection^[59]. Significant positive correlations between ECs apoptotic index and nuclear ECs NF- κ B index, and between leukocyte apoptotic index and intravascular leukocyte NF- κ B index in CM brains were verified. These findings conferred that apoptotic changes in intravascular leukocytes and ECs in CM are potentiated by NF- κ B p65 signaling^[59].

Expression of glial and neuronal NF- κ B p65 and caspase-3 had no association with age, leucocyte count, anemia, hospitalization, last parasite count, and time to death^[59].

The expression of NF- κ B and apoptosis in severe *falciparum* malaria were studied by Viriyavejakul *et al.*^[60] in liver tissues from patients who had died with associated hyperbilirubinaemia, and those without hyperbilirubinaemia, compared to patients who had died from accidents. They reported significantly higher expression of cleaved caspase-3 in lymphocytes and Kupffer cells in the non-hyperbilirubinaemia group, compared to the normal controls, as well as significantly increased lymphocytes and Kupffer cells apoptosis, and lymphocytes and Kupffer cells NF- κ B p65 expression. Besides, they recorded presence of significant positive correlation between NF- κ B p65 and cleaved caspase-3 expression in portal lymphocytes and Kupffer cells. A significant positive correlation was also demonstrated between NF- κ B p65 and cleaved caspase-3 expression with total bilirubin in lymphocytes and Kupffer cells. Additionally, the expressions of NF- κ B p65 and cleaved caspase-3 were verified to positively correlate with hyperplastic Kupffer cells. The score of portal tract inflammation also showed significant positive correlation with the expression of NF- κ B p65 and cleaved caspase-3. Non-significant associations were documented between age, gender, duration of pyrexia before hospitalization, liver function tests, and level of parasitaemia, and both portal lymphocytes and Kupffer cells expression of cleaved caspase-3 and NF- κ B p65^[60].

Theileria parva

In cattle, *T. parva* replicates in the cytoplasm of lymphocytes, induces lymphoblastoid transformation, and causes clonal expansion of the infected cell. This autocrine growth caused by induced expression of IL-2 and IL-2 receptor because of the persistent translocation of NF- κ B to the nucleus, can be reversed by eradicating the parasite^[61]. In *T. parva*-infected cloned bovine T cells, higher I κ B α levels and lower I κ B β levels were detected, compared to uninfected cells, in response to specific anti-I κ B α and anti-I κ B β antibodies. *Theileria* provoked continuous degradation of both I κ B α and I κ B β . I κ B α re-accumulated to high levels in infected cells, denoting that *T. parva* did not affect the normal NF- κ B-mediated positive feedback loop, while maintaining low cytoplasmic I κ B β levels. The use of BW720c (a hydronaphthoquinone derivative lethal to *T. parva*) restored normal I κ B β levels. In addition, treatment of *T. parva*-infected cells with pyrrolidine dithiocarbamate inhibited I κ B degradation, in contrast to the anti-oxidant N-acetylcysteine that showed no effect on I κ B levels or NF- κ B activation^[62].

Helminths

Fasciola hepatica

Hamilton *et al.*^[63] studied the effects of *F. hepatica* tegumental antigen on the activation and function of

DCs in mice, and reported that the antigen significantly suppressed nitrite, TNF- α , IL-6, IL-10, and IL-12 p70 levels, and the expression of cell surface marker of CD40, CD80, and CD86 in DCs matured with TLR and non-TLR ligands. They also documented suppression of NF- κ B p65 in mature DCs, which can justify the reduction in pro-inflammatory cytokine levels, implying that the tegumental antigen of *F. hepatica* has potent anti-inflammatory activities and a possible beneficial effect as an anti-inflammatory component^[63].

Clonorchis sinensis

Previous studies reported that contact of *C. sinensis* adult parasites and their ESPs with bile ducts and adjacent hepatic tissues, as well as the subsequent oxidative stress and chronic inflammation aggravate epithelial hyperplasia, periductal fibrosis, and cholangiocarcinogenesis^[64,65]. Pak *et al.*^[66] investigated two redox-active proteins induced by ESPs; peroxiredoxin 6 (Prdx6) and NF- κ B, in murine clonorchiasis. The authors reported that in ESPs-treated cholangiocarcinoma cells, the generation of intracellular free-radicals was inhibited by overexpression of Prdx6 through inhibition of iNOS and NADPH oxidase 2, significantly decreasing inflammation; and that overexpression of NF- κ B suppressed Prdx6 transcription levels. They also demonstrated that immunoreactive NF- κ B and Prdx6 distribution was more marked in the liver in initial infection stages, and that their immunopositive staining extent and intensity in infected mice correlates with both duration and severity of infection. These findings suggest that the increased levels of NF- κ B and Prdx6 in clonorchiasis are linked to massive histopathological changes^[66].

Using cholangiocarcinoma cell lines, Pak *et al.*^[67] investigated the effects of ESPs on cellular migration and invasion, and documented significantly increased concentration-dependent expression and activity of matrix metalloproteinase-9 (MMP-9) enzymes through augmented NF- κ B signaling. Besides, ESPs induced I κ B α degradation and phosphorylation, and provoked nuclear translocation of NF- κ B p65, controlled by extracellular signal regulated protein kinase 1 and 2 activity. These findings denote that both ESPs-provoked MMP-9 activity and stimulated NF- κ B and I κ B α are associated with a progressive course of cholangiocarcinoma^[67]; since MMP-9, which is involved in the degradation of the extracellular matrix, is well-recognized to have a principle effect on invasion of malignant cells and metastasis^[68].

Opisthorchis viverrini

Infection with *O. viverrini* elicits inflammation-mediated oxidative and nitrosative DNA damage, which predisposes to the development of cholangiocarcinoma^[69]. In 2005, Pinlaor *et al.*^[70] evaluated the effects of an *O. viverrini*-derived antigen on expression of TLRs using RAW 264.7 macrophage

cell line, and reported that the antigen provoked TLR2 expression. They also documented dose-dependent expression of NF- κ B, cyclooxygenase-2, and iNOS, in RAW 264.7 cells, in response to *O. viverrini* antigen.

In 2006, Pinlaor *et al.*^[71] investigated the preventive effect of the anti-helminthic drug praziquantel (PZQ) on 8-nitroguanine and 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) formation, and iNOS expression in hamsters infected with *O. viverrini*. Apparently, the PZQ treatment did not totally eradicate the parasites, but markedly decreased inflammatory cell infiltration, totally reduced *O. viverrini*-provoked production of 8-nitroguanine and 8-oxodG in biliary cells, and significantly decreased hepatic 8-oxodG level in biliary epithelium. There was an associated reduced NF- κ B and iNOS expression in biliary cells, and significantly decreased levels of nitrite and nitrate in plasma and liver of *O. viverrini*-infected treated hamsters, compared to controls.

In 2010, Laothong *et al.*^[72] documented that melatonin can guard against cholangiocarcinoma provoked by *Opithorchis* infection, through decreasing nitrosative and oxidative DNA damage through initiating the activity of erythroid 2-related factor 2 (Nrf2), as well as inhibiting NF- κ B activity. The authors studied the protective effects of oral melatonin and reported decreased levels of 8-nitroguanine and 8-oxodG in inflammatory cells and biliary epithelium, and 3-nitrotyrosine in the liver, and decreased levels of nitrate/nitrite, and cytokeratin 19 (CK-19; used as a marker to assess proliferation of bile ducts) and heme oxygenase-1 expression, in hamsters infected with *O. viverrini*. In addition, they documented dose-dependent decreases in alanine transaminase, 8-isoprostane, and vitamin E levels in plasma, and in nitrate/nitrite level in liver homogenates, in melatonin-treated hamsters. Besides, IL-1 β , TNF- α , and oxidant-generating genes (NF- κ B, cyclooxygenase-2, and iNOS) showed dose-dependent reductions in mRNA expression, in treated hamsters. They also demonstrated a dose-dependent increased expression of anti-oxidant genes (erythroid 2-related factor 2 and manganese superoxide dismutase), compared to *O. viverrini*-infected untreated hamsters.

***Schistosoma* spp.**

In 2015, Ashour *et al.*^[73] studied NF- κ B activity in murine colonic schistosomiasis *mansoni* and documented marked increase in TLR2 expression and NF- κ B positive cells in all colonic cells, granulomas, and lymphoid follicles, compared to uninfected mice; and recommended that TLR2 and NF- κ B could be targeted to treat colonic schistosomiasis.

The effect of genistein on inhibiting the activity of NF- κ B in *S. japonicum* hepatic granuloma and fibrosis was evaluated by Wan *et al.*^[74] who demonstrated

amelioration of liver pathology. This included regression of granulomatous inflammation, inhibited mRNA expression of NF- κ B p65, I κ B- α , TNF- α , monocyte chemotactic protein-1, IL-1 β , IL-4, and IL-10 in liver tissue during early phase of infection. Significant reductions in the contents of transforming growth factor- β (TGF- β) and α -smooth muscle actin (α -SMA) in liver tissue in late infection, were also recorded in genistein-treated mice, compared to infected mice.

Besides, the hepatoprotective effects of NF- κ B inhibition on murine schistosomiasis *mansoni* were investigated by Alhusseiny *et al.*^[75] who evaluated the effects of the NO donor isosorbide-5-mononitrate (IS-5-MN) and the NF- κ B inhibitor vinpocetine, as adjuvants to praziquantel on murine schistosomiasis *mansoni*. The authors documented significantly reduced NF- κ B p65 expression following vinpocetine as monotherapy and when combined with IS-5-MN. This NF- κ B inhibitory effect partially explained the significantly decreased diameter and count of granulomas, in response to vinpocetine monotherapy^[76], as a result of decreased IFN- γ activity^[77], and decreased TNF and delayed granuloma formation^[78], respectively.

***Echinococcus* spp.**

The role of NF- κ B in controlling local and systemic inflammatory and immunological responses in patients with pulmonary hydatidosis either for the first time or in patients with rebound infection was investigated by Tilioua *et al.*^[79]. The authors studied NF- κ B and iNOS expression in peripheral blood mononuclear cells (PBMC) and in peri-cystic layer, and assessed NO and TNF- α in plasma samples. They verified high expression of NF- κ B and iNOS in both PBMCs and peri-cystic histiocytes, as well as elevated NO and TNF- α levels in patients with primary infection. In contrast, they documented reduced expression of NF- κ B and iNOS, and reduced NO and TNF- α levels in patients with rebound hydatidosis denoting that NF- κ B suppressed activity is associated with defective immune response and helps the parasite evade host immunity in rebound patients^[79].

CONCLUDING REMARKS

1. Data presented here evidently denotes that host NF- κ B activation is associated with the development of severe pathology following parasitic infections due to high levels of cytokines and inflammatory mediators.
2. Parasites can manipulate NF- κ B; cleavage, degradation, or reduced levels of p65 subunit were verified in response to *G. lamblia*, *T. gondii*, and *L. amazonensis*, respectively.
3. Signaling activity of NF- κ B was proved to be associated with anti-apoptotic effects in *C. parvum* and *T. gondii*.
4. Activation of NF- κ B signaling was linked to beneficial consequences in *E. histolytica* and *L. major*.

5. Inhibition of NF- κ B activity can alleviate inflammation caused by *T. vaginalis* infection, lymphoblastoid transformation in *T. parva*, and histopathological changes induced by *S. japonicum*, *S. mansoni*, *C. sinensis*, and *O. viverrini*.
6. Targeting NF- κ B signaling pathways could be a promising approach for ameliorating tissue damage caused by the inflammatory mediators, by precise control between harmful and beneficial effects of host NF- κ B activation.
7. Future in-depth studies are crucial to explore the effect of NF- κ B activation and inhibition on protozoan and helminthic parasitic infections, and to develop NF- κ B cell-specific agents that inhibit NF- κ B activity only in target cells, without inducing toxicity or affecting innate immunity.

Author contribution: Alhusseiny, SM conceived the review topic, searched for information, and wrote the manuscript. El-Beshbishi, SN helped in the review design, critically revised the manuscript for intellectual content and approved the final version for publication.

Conflict of interest: None.

Funding statement: None.

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