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Review

The role of nuclear factor-κB in the development of autoimmune diseases: a link between genes and environment

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Although autoimmune diseases are relatively common, mechanisms that lead to their development remain largely unknown. Nuclear factor-κB (NF-κB), as a key transcription factor involved in the regulation of immune responses and apoptosis, appears to be a good candidate for studies on the pathogenesis of autoimmunity. This review presents how perturbations of the NF-κB signaling pathway may contribute to self-tolerance failure, initiation of autoimmune inflammatory response as well as its persistent maintenance and therefore to the development of common autoimmune diseases including rheumatoid arthritis, multiple sclerosis, type 1 diabetes mellitus, thyroid autoimmune diseases, systemic lupus erythematosus as well as inflammatory bowel diseases and psoriasis. A special emphasis is put on the genetic variations in the NF-κB related genes and their possible association with susceptibility to autoimmune diseases, as well as on the therapeutic potential of the NF-κB targeted strategies in the treatment of autoimmunity.

Keywords: nuclear factor-κB (NF-κB), autoimmune diseases, genetic polymorphism, NF-κB targeted strategies

INTRODUCTION

Since its discovery in 1986, the nuclear factor-κB (NF-κB) has attracted widespread attention due to the number of stimuli that can activate it and the variety of genes and biological responses that it controls. There is also a growing amount of evidence that both enhanced and impaired activation of the NF-κB pathway may play a key role during the development of common human diseases. Therefore, the NF-κB transcription factor and related proteins constitute interesting targets for design and testing of novel drugs (Yates & Górecki, 2006).

In this article we present the current state of knowledge regarding confirmed and potential mechanisms by which the NF-κB signaling pathway may be involved in the development of autoimmune diseases. First, we provide a brief description of the NF-κB family of transcription factors and mechanisms of their activation. Next, we focus on the role of the NF-κB signaling pathway in the immune system and suggest how its disturbance may be implicated in the initiation and progression of autoimmunity at different stages. Subsequently, we summarize the *in vitro* and *in vivo* data demonstrating involvement of NF-κB proteins in the development of common autoimmune and chronic inflammatory diseases, including rheumatoid arthritis, multiple sclerosis, type 1 diabetes mellitus, thyroid autoimmune diseases, systemic lupus erythematosus as well as inflammatory bowel diseases and psoriasis. Afterwards we describe possible associations of

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Abbreviations: AIH, autoimmune hypothyroidism; APC, antigen presenting cells; CD, Crohn's disease; CIA, collagen induced arthritis; DC, dendritc cells; EAE, experimental allergic encephalomyelitis; GD, Graves' disease; IBD, inflammatory bowel diseases; IRAK, IL-4 receptor-associated kinase; IκB, inhibitor κB; IKK, inhibitor κB kinase; LPS, lipopolysaccharide; MS, multiple sclerosis; NEMO, NF-κB essential modulator; NF-κB, nuclear factor κB; NLR, NOD-like receptor; NOD, nucleotide-binding oligomerization domain; ODN, oligodeoxynucleotides; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; SNP, single nucleotide polymorphism; SUMO, small ubiquitin related modifier; T1DM, type 1 diabetes mellitus, TLR, Toll-like receptors, TRAF, TNF, receptor-associated factor; UC, ulcerative colitis.

the polymorphisms in the NF-κB related genes with the prevalence of autoimmune disorders in different populations. Finally, we present the therapeutic potential of the NF-κB targeted strategies in the treatment of autoimmune diseases.

THE NF-κB FAMILY AND MECHANISMS OF ITS ACTIVATION

The NF-κB family

In mammals the NF-κB family of transcription factors consists of two groups of proteins which differ in their structure, function and way of synthesis. The first group includes p105 and p100 which are synthesized as large polypeptides and as the result of limited proteolysis generate short active molecules (p50 known as NF-κB1 and p52 known as NF-κB2, respectively). While p50 is generated by constitutive p105 processing, p100 proteolysis is tightly regulated through its inducible phosphorylation by NIK (NF-κB inducing kinase) and its polyubiquitination (Sun & Ley, 2008). The second NF-κB subfamily comprises p65 (RelA), c-Rel (Rel) and RelB proteins that do not originate from precursors and possess C-terminal transcriptional activation domains. Members of both NF-κB subfamilies are characterized by the presence of a Rel homology domain which contains a nuclear localization sequence and is involved in DNA binding, dimerization and interaction with κB inhibitory proteins (IκB — described in the following sections). Generation of transgenic mice deficient for each Rel protein revealed that the individual NF-κB family members have distinct, non-redundant functions. Furthermore, the NF-κB proteins can form homo- or heterodimers, which in response to specific stimuli may have different effects on target gene transcription. The first heterodimer, identified as a NF-κB, consisted of p50 and p65 subunits (Siebenlist *et al.*, 1994). This heterodimer is known to be rapidly activated and with time can be replaced in promoters of target genes by other, slowly activated dimers e.g. p52/RelB. In turn, the RelB/p65 complex was found to act as an inhibitor, since it cannot bind efficiently to the conventional NF-κB sites (Marienfeld *et al.*, 2003). As a rule, when complexed with each other, the p50/p50 and p52/p52 homodimers that lack transcriptional activation domains act as transcriptional repressors. However, in the presence of particular coactivators like BCL-3 (an IκB-like protein, product of the proto-oncogene *BCL-3*), the p50/p50 homodimer was found to activate transcription (Zhang *et al.*, 2007). The distribution of NF-κB transcription factors can differ between tissues (for instance, whereas the p50/p65 heterodimer is expressed ubiquitously, the p52, c-Rel and RelB are expressed specifically in lymphoid cells and tissues) and the intercellular balance between different NF-κB dimers determines which complex will bind the target DNA sequence.

Mechanisms of NF-κB activation

In unstimulated cells, NF-κB dimers are sequestered in the cytoplasm thanks to their association with κB inhibitors (IκBs, e.g. IκBα, IκBβ, IκBε and others). The IκB proteins are composed of a central ankyrin repeat region that interacts with the nuclear localizing signals of the Rel homology domain and in this way prevents nuclear translocation of NF-κB, and a N-terminal regulatory domain responsible for their inducible degradation. The p105 and p100 (NF-κB1 and NF-κB2 precursors) that posses ankyrin repeat regions in their C-terminal parts can also act as IκBs. Moreover, alternate promoter usage of the *nfκb1* gene can generate an mRNA encoding only the C-terminal IκB-like part of p105, a protein termed IκBζ (Wegener & Krappmann, 2008).

Specific (e.g. lipopolysaccharide — LPS, cytokines such as tumor necrosis factor α — TNFα or interleukin-1 — IL-1) as well as unspecific (e.g. γ and UV radiation, oxidative stress) activating signals can trigger the transduction pathways leading to the dissociation of NF-κB from IκB proteins. The first step of this process involves activation of the IκB kinases (IKK). The IKK complex consists of two catalytic subunits: IKKα (IKK1) and IKKβ (IKK2) as well as a regulatory subunit: NEMO (NF-κB essential modulator, IKKγ). IKKβ is crucial for IκBα phosphorylation triggered by such stimuli as TNF-α and IL-1. Engagement of TNF-α and IL-1 receptors on the cell surface leads to the activation of the downstream kinases which are responsible for the direct phosphorylation of the IKKβ activation loop (reviewed by Lee & Hung, 2008). Subsequently, IKKβ phosphorylates serine 32 and 36 residues in the N-terminal part of the IκBα protein, thus creating a binding site for the subunits of the ubiquitin ligase complex and resulting in the rapid polyubiquitination of IκBα followed by its degradation in the 26S proteasome. The agonist-induced degradation of IκBβ and IκBε proceeds in a similar way (Li & Verma, 2002). Dissociation of IκB exposes the nuclear localization signal in NF-κB proteins and leads to their nuclear translocation and binding to the promoters of target genes. Interestingly, one of the genes up-regulated by NF-κB is a gene encoding IκBα, which migrates to the nucleus and removes NF-κB from its cognate DNA-binding sites and down-regulates a set of immediate early target genes (Karin & Ben Neriah, 2000).

This signaling pathway which leads to the degradation of IκBα and release of the p50/p65 and p50/c-Rel dimers (referred to as the "classical" or

"canonical") was found to be essential for immune and inflammatory responses, as well as for the promotion of cell survival. Other stimuli (e.g. lymphotoxin β1, B-cell-activating-factor — BAFF and CD40 ligand) trigger the so-called "alternative" or "non-canonical" pathway that regulates a distinct subset of target genes, mainly involved in adaptive immunity, including formation of secondary lymphoid organs and development of B cells. This pathway does not involve NEMO/IKKγ and requires IKKα. IKKα, activated by NIK, induces a slow processing of p100 to p52 and results in the nuclear translocation of p52/RelB dimers (Senftleben *et al.*, 2001).

The third mechanism of NF-κB activation, described by Rice and coworkers involves complete proteasomal degradation of p105. As it was mentioned above, p105 may also act as IκB and retain other subunits (mainly p50) in the cytoplasm. Agonists such as TNF-α, IL-1 or LPS may trigger phosphorylation of the p105 PEST region (sequence enriched in proline — P, glutamate — E, serine — S, threonine — T) by the classical IKKα/IKKβ/NEMO complex. This induces polyubiquitination of p105, its subsequent degradation and finally, release and nuclear translocation of p50 homodimers (Rice *et al.*, 1992).The coordinated degradation and resynthesis of the IκB proteins results in the oscillations in the NF-κB activity.

THE ROLE OF NF-κB IN AUTOIMMUNE RESPONSES

The immune system uses several mechanisms in the prevention of autoimmunity. Tolerance towards self-antigens is generated at two levels by means of central and peripheral mechanisms. In short, central tolerance is developed during lymphopoesis in thymus and bone marrow. In thymus, T cell receptors are exposed to the antigens from self-molecules and if such an interaction results in strong and high affinity binding, the lymphocyte is detected as autoreactive and is eliminated by apoptosis (this is called a negative selection). Similarly, developing B cells receive deletional signals from self-antigens in the bone marrow, but the selection process continues in germinal centers of peripheral lymphoid tissues as well. Since not all self-antigens are available for efficient negative selection and some autoreactive lymphocytes can be found in the periphery, mechanisms of peripheral tolerance such as ignorance, anergy, clonal deletion or action of regulatory T cells (reviewed by Goodnow *et al.*, 2005) help to prevent activation of autoreactive cells.

The existence of autoantibodies and autoreactive T or B cells is therefore a natural phenomenon and is not sufficient to elicit autoimmune disease. Both environmental and genetic factors may contribute to the tolerance failure and to the development of autoimmune responses. Here we present how the NF-κB signaling pathway can be implicated in the initiation and progression of autoimmunity at different stages, including: breakdown of central and peripheral tolerance, development of initial autoimmune inflammatory response as well as its persistent maintenance.

NF-κB and tolerance towards self-antigens

An idea that NF-κB-related molecules are involved in the formation of central and peripheral tolerance came from the analysis of animal models. Mice deficient in *nfkb2* (*nfkb2*$^{-/-}$), as well as mice with *relb* knockout (*relb*$^{-/-}$), display defective thymic organogenesis and a reduced number of thymic medullary epithelial cells that results in impaired negative selection, increased numbers of autoreactive effector/memory T cells in the periphery and multi-organ inflammation (Akiyama *et al.*, 2005; Zhang *et al.*, 2006). Similar disorganization of thymic function was observed in the *aly/aly* mice, which carry a functional mutation in the NF-κB inducing kinase (NIK) that makes it unable to bind IKKα and thereby to phosphorylate p100 (Tamura *et al.*, 2006).

Disruption of central tolerance *via* targeting NF-κB proteins can result not only in the increased number of autoreactive T cells in periphery, but also in an increased activity while they face self-antigens. T cells isolated from animals with a double *bcl-3* and *nfkb2* knockout, in the presence of syngenic antigen presenting cells (APCs), proliferate at a significantly higher rate than T cells from wild type animals and even at a higher rate than T cells from the two single knockouts (Zhang *et al.*, 2007).

Several lines of evidence indicate that NF-κB signaling pathways involved in the regulation of cell survival and activation in thymus can also be crucial for development of peripheral tolerance. Functional studies revealed that T lineage-specific inhibition of NF-κB activation *via* deletion of IKK2 interferes with the generation of regulatory and memory T cell subsets (Schmidt-Supprian *et al.*, 2003). Similarly, simultaneous knockout of *relb* and TRAF6 (TNF receptor-associated factor 6, one of the NF-κB pathway downstream proteins) results in the reduced number of CD4+CD25+ regulatory T cells (Thomas, 2005). In turn, inhibition of NF-κB during APCs differentiation results in a weak stimulation of autoreactive T cells that leads to their anergy (Hernandez *et al.*, 2007). Additionally, APCs from *relb*$^{-/-}$ mice overproduce interleukin-1β (IL-1β) (Caamano *et al.*, 1999), whereas from *c-rel*$^{-/-}$ mice — IL-6 (Beinke & Ley, 2004) — two cytokines crucial for differentiation of Th17 cells — subset of CD4+ effector cells involved

in the development of organ specific autoimmunity in murine models and probably also in humans (Romagnani, 2008).

These data suggest that abnormalities of the NF-κB pathway resulting in defects in the interaction of APCs and thymocytes that can interfere with the normal process of negative selection, as well as impaired action of regulatory T cells, facilitate release and survival of autoreactive T cells in the periphery, where subsequent environmental events more readily trigger autoimmune disease.

NF-κB and initiation of autoimmune inflammation

Infection constitutes an excellent example of an environmental stimulus implicated in the disruption of peripheral tolerance and initiation of autoimmunity that acts *via* NF-κB signaling pathways. On the other hand, animals deficient in particular NF-κB-related proteins (e.g. p50) are more susceptible to intracellular and extracellular Gram-positive bacterial infections (Ishikawa *et al.*, 1998).

Upon infection, pathogenic microorganisms activate NF-κB transcription factors *via* triggering the Toll-like receptors (TLRs) on macrophages, DCs and other cells of the innate immune system that together with anatomical barriers act as the first line of defense. TLRs belong to the family of pattern recognition receptors that identify invading pathogens by the recognition of invariant structures called pathogen-associated molecular patterns such as: LPS, bacterial lipoprotein, lipoteichoic acid, bacterial unmethylated CpG DNA and others (reviewed by Medzhitov, 2007). As a result of pathogen engagement, TLRs form homodimers and recruit adapter molecules such as MyD88 (myeloid differentiation primary response gene 88). Subsequently, the death domain of MyD88 recruits downstream kinase IRAK (IL-1 receptor-associated kinase) to the receptor complex. This results in IRAK autophosphorylation and dissociation from the complex, recruitment of TRAF6 and activation of downstream kinases such as TAK1 (transforming-growth-factor-β-activated kinase) associated with adaptor TAB molecules. TAK1 is capable of IKK activation that results in IκB phosphorylation and dissociation, release of NF-κB and its translocation to the nucleus (Fig. 1). In turn NF-κB activates transcription of inflammation-related genes: cytokines, chemokines, immune receptors and other molecules that are important components of the innate immunity responses and are required for the migration of inflammatory and phagocytic cells into areas of infection or injury.

Apart from the typical surface localization, some members of the TLR family, including TLR 3, 7, 8 and 9, can be found in the endoplasmatic reticulum, endosomes and lysosomes. These receptors, designed to recognize bacterial or viral nucleic acid components, can be also activated by endogenous DNA and RNA and *via* activation of the NF-κB pathway induce a typical autoimmune reaction (Hurst & Landenberg, 2008). Moreover, in some autoimmune diseases, ectopic expression of TLRs in different cells (e.g. presence of TLR3 in thyreocytes observed in autoimmune hypothyroidism) enables induction of a local immune response *via* targeting NF-κB signaling pathway (Harii *et al.*, 2005).

Interestingly, gastric and intestinal cells were found to be largely deficient in TLR signaling and must rely on alternative systems of pathogen detection, such as Nod-like receptors (NLRs). The NLR family represents another group of intracellular and host-specific cytosolic pattern recognition receptors containing a nucleotide binding oligomerization domain (NOD). The first NLRs reported to have a direct function as intracellular pattern recognition molecules were NOD1 and NOD2 receptors that recognize components of bacterial cell wall peptidoglicans (muramyl dipeptide and iE-DAP dipeptide, respectively). Binding of NLRs by pathogens induces a signal transduction cascade involving RIP2 kinase responsible for IKK activation, finally leading to the release of NF-κB, its nuclear translocation and activation of pro-inflammatory genes in epithelial cells (Franchi *et al.*, 2006).

Breakdown of vascular and cellular barriers in the course of pathogen-induced inflammation, seems to be the main mechanism by which TLRs *via* activation of NF-κB lead to the breakdown of peripheral tolerance. Self-antigens (unavailable for the immune system until that moment) are phagocytosed and transported by APCs to the draining lymph nodes and finally presented to the autoreactive T cells. Presentation of self-antigens to the autoreactive T cells leads to their activation and subsequent development of cellular or humoral autoimmune responses that result in the damage of self tissues. Since its first discovery in 1986 as a nuclear factor indispensable for immunoglobulin κ light chain transcription in B cells, NF-κB has been found to play a critical role in both T and B cell development, activation and function. Subsequently, deficiency and/or disturbance of particular NF-κB-related proteins can lead to several abnormalities in both cellular and humoral responses, other than autoimmunity (reviewed by Hayden *et al.*, 2006) which are not the focus of this review.

NF-κB and maintenance of autoimmune inflammation

Development of autoimmune diseases requires coordinated expression of a number of genes that are involved in the activation, migration and ef-

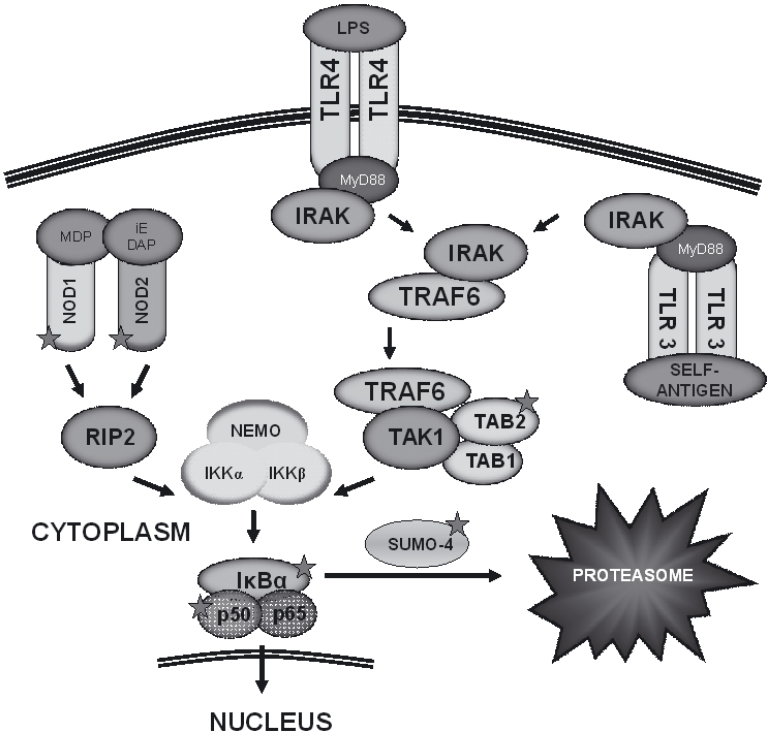

Figure 1. A simplified diagram presenting activation of the nuclear factor κB (NF-κB) signaling pathway in the course of infection.
Upon activation *via* bacterial products (e.g. lipopolysaccharide — LPS), Toll-like receptors (TLR) form homodimers and recruit adapter molecules such as MyD88 (myeloid differentiation primary response gene 88). MyD88 recruits IRAK (IL-1 receptor-associated kinase) that results in IRAK autophosphorylation and dissociation from the complex, recruitment of TRAF6 (TNF receptor-associated factor 6) and activation of downstream kinases such as TAK1 (transforming-growth-factor-β-activated kinase) associated with adaptor TAB molecules. TAK1 activates the κB inhibitor kinases (IKK) complex that consists of two catalytic subunits: IKKα, IKKβ and a regulatory subunit: NEMO (NF-κB essential modulator). IKK complex phosphorylates κB inhibitors (e.g. IκBα) resulting in the rapid polyubiquitination of IκB followed by its proteasomal degradation, release of NF-κB dimers, their nuclear translocation and activation of the pro-inflammatory genes. Small ubiquitin related modifier 4 (SUMO-4) is one of the proteins involved in the IκB degradation. Apart from the typical surface localization, some members of TLR family (e.g. TLR3) can be find in the endoplasmatic reticulum, endosomes and lysosomes and activated by endogenous DNA and RNA and *via* activation of NF-κB pathway induce a typical autoimmune reaction. Gastric and intestinal epithelial cells are deficient in TLR signaling and rely on the alternative systems of pathogen detection, such as intracellular NOD (nucleotide-binding oligomerization domain)-like receptors. The NOD-like receptors recognize components of bacterial cell wall peptidoglycans (e.g. muramyl dipeptide — MDP and iE-DAP dipeptide). NOD binding induces a signal transduction cascade involving RIP2 kinase responsible for IKK activation. Stars mark proteins whose genetic variants are associated with development of autoimmune diseases.

fector functions of inflammatory cells. These include many genes activated by the NF-κB signaling pathway. Since some of the NF-κB target genes (such as IL-1 or TNFα) may also serve as NF-κB activators, the NF-κB feedback can rapidly enhance the inflammatory response even in the presence of low amounts of the inflammatory inducing agent (a self-antigen). This system also bears a danger to induce or sustain the inflammatory response even in the absence of the inducing factors.

Apoptosis represents a physiological process critical for development and selection of both: T and B cells involved in suppression of the inflammatory response. NF-κB transcription factors act as critical regulators of the apoptotic program in diverse cell types, however, whether they promote or inhibit apoptosis depends on the specific cell type and the type of inducer. It is also supposed that the dual function of NF-κB depends on the relative, intracellular levels of particular subunits.

In general, NF-κB activation is required for protection of immune cells from induction of apoptosis by various stimuli. *In vitro* studies showed that NF-κB is essential in protection of T cells from induction of apoptosis by mitogens, Fas, TNFα as well as TCR engagement. In turn, B-cells from *c-rel*$^{-/-}$ mice are more susceptible to apoptotic stimuli (Hilliard *et al.*, 2002), whereas NF-κB inhibition with different agents act as inductor of apoptosis (reviewed by Liang *et al.*, 2004).

On the other hand, NF-κB plays an essential role in promoting T cells and double positive thy-

mocyte apoptosis during generation of central tolerance in the thymus but also in the limitation of antigen-specific clonal expansion of T cells. In general, activation of the TCR also delivers signals required to kill activated T cells at the end of the immune response (so called activation-induced cell death), that depends on a functional FasL/Fas signal transduction pathway. Indeed, impaired elimination of activated T cells appears to be the primary defect in mice that are homozygous for the mutation of FasL or Fas (known as *gld*/*gld* and *lpr*/*lpr* mice) and results in the spontaneous development of an autoimmune syndrome characterized by production of autoantibodies, glomerulonephritis and arthritis. Interestingly, T-cell specific inhibition of NF-κB by nondegradable IκBα mutant can partly rescue the ongoing autoimmune disease, probably due to the activation of other cell death pathways upon TCR triggering (Vallabhapurapu *et al.*, 2001). In this case, the uncorrected intrinsic B cell defect, which results from impaired elimination of autoreactive B cells *via* FasL/Fas-mediated apoptosis, is probably responsible for only partial correction of the autoimmune defect.

To sum up, there are at least three possible stages in development of autoimmunity when defects of the NF-κB signaling pathway can occur to be causative: (1) disturbance of central and peripheral tolerance formation, leading to the "leakage" and uncontrolled action of autoreactive T cells in the periphery; (2) disruption of peripheral tolerance *via* targeting TLR receptors and initiation of primary autoimmune inflammation; (3) maintenance of the autoimmune response even in the absence of the inducing factors due to the disturbed apoptosis of autoreactive and activated cells. The next section will present examples of autoimmune or chronic inflammatory disorders in which some abnormalities of the NF-κB signaling pathway were described.

NF-κB SIGNALING PATHWAY IN THE DEVELOPMENT OF AUTOIMMUNE DISEASES

So far, there is no direct evidence that mutation or knockout of NF-κB related genes leads to the spontaneous development of autoimmunity. However, as it was mentioned above, animals deficient for different NF-κB related proteins present some abnormalities also observed in patients with autoimmune and chronic inflammatory diseases. Mice lacking *nfkb1* were found to be highly susceptible to bacterial infections and exhibit chronic inflammation, whereas *nfkb2* knockout leads to increased numbers of autoreactive T cells in the periphery and their enhanced activity (Akiyama *et al.*, 2005). Not surprisingly, the *nfkb1*/*nfkb2* double deficient mice suffer from both developmental and immunological abnormalities. $Relb^{-/-}$ mice, apart from the disrupted lymphoid architecture, develop a T-cell mediated multiorgan inflammation which results in their death by 8 to 12 weeks after birth. T and B cells from *c-rel* deficient mice present impaired responsiveness to mitogenic stimuli and disturbed production of immunoglobulins (Hilliard *et al.*, 2002). These studies indicate a key role of the NF-κB pathway in development of tolerance towards self-antigens and control of inflammatory and autoimmune responses in animals and suggest its potential involvement in the development of autoimmunity in humans. Indeed, recent years have provided some evidence that disturbed NF-κB signaling may be implicated in the pathogenesis of human autoimmune and chronic inflammatory diseases, including rheumatoid arthritis, multiple sclerosis, type 1 diabetes mellitus, thyroid autoimmune diseases, systemic lupus erythematosus as well as inflammatory bowel diseases and psoriasis. Although in most cases, activation of the NF-κB signaling pathway in the course of these conditions is associated with regulation of inflammatory response and/or cell apoptosis, there are also some disease-specific mechanisms in which NF-κB contributes to their development.

Rheumatoid arthritis

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease which is characterized by progressive joint destruction caused by the chronic inflammation of the synovial lining. Infiltration of this tissue with immunocompetent cells and proliferation of synovial fibroblasts leads to the formation of pannus tissue which invades and destroys the articular cartilage and subchondral bone. Both *in vitro* and *in vivo* studies indicate an important role of NF-κB signaling in the development and progress of in RA. Patients with RA present constitutively high serum levels of pro-inflammatory cytokines, including: TNFα, IL-1 and IL-6 which are known to be NF-κB target genes, suggesting activation of this signaling pathway in the course of disease. Indeed, activated forms of p50 and p65 were found in the nuclei of cells from synovial lining in RA patients, whereas in the nuclei of cells from normal synovium these transcription factors were not detected (Handel *et al.*, 1995). Furthermore, expression of p50 in synovial tissue was found to be highest at the cartilage-pannus junction — a site most likely to be associated with joint erosion (Benito *et al.*, 2004). Apart from the increased concentration of the active NF-κB in synovium, its binding to DNA was found to be much stronger in RA compared to patients with osteoarthritis (Han *et al.*, 1998).

Evidence for the pathogenic role of NF-κB in the development of RA also comes from animal

models of inflammatory arthritis. In murine collagen-induced arthritis (CIA), increased synovial NF-κB binding precedes development of clinical joint involvement and gradually increases during the evolution of the disease (Han *et al.*, 1998). In turn, mice with *c-rel* or *nfkb1* knockouts were found to have impaired cellular and humoral immunity to type II collagen and largely failed to develop CIA. Interestingly, c-Rel-deficient mice show a normal response in an acute destructive arthritis (induced with methylated bovine serum albumin/IL-1), suggesting that c-Rel is not required for the destructive phase of joint disease. In contrast, *nfkb1*$^{-/-}$ mice were refractory to the induction of acute arthritis, showing that this subunit is essential for the local joint inflammation and destruction. These data also suggest that NF-κB transcription factors can play distinct roles in the pathogenesis of inflammatory arthritis (Campbell *et al.*, 2000).

IKKβ is another member of the NF-κB cascade implicated in synovial inflammation. In rats, local overexpression of wild-type IKKβ in joints results in clinical signs of arthritis, whereas intra-articluar administration of dominant negative IKKβ mutant, dramatically reduces ongoing inflammation, suggesting that inhibition of NF-κB activation *via* targeting the IKK complex may constitute an attractive strategy for the treatment of chronic autoimmune arthritis (Tak *et al.*, 2001). Concomitantly, local administration of highly specific IKKβ inhibitor, a NEMO-binding domain peptide, was found to reduce arthritis activity and bone destruction in rats, and to inhibit production of pro-inflammatory cytokines in macrophages and synoviocytes from patients with RA (Tas *et al.*, 2006). Moreover, local suppression of NF-κB by either sequesteration of NF-κB dimers in an inactive cytoplasmatic complex or by the use of proteasomal inhibitors (described in the following sections) resulted in increased apoptosis in the synovial membrane and in subsequent reduction of undesirable pannus proliferation. These results indicate that activation of NF-κB may protect synoviocytes from apoptosis and thus provide a potential link between inflammation and hyperplasia. Unexpectedly, the severity of arthritis was also inhibited significantly in the contralateral, untreated joints, indicating beneficial systemic effects of local suppression of NF-κB (Miagkov *et al.*, 1998).

Multiple sclerosis

Multiple sclerosis (MS) is a chronic inflammatory illness affecting the central nervous system (CNS). The main pathological feature of MS is destruction of myelin sheaths with ensuing damage to the neural axons (Noseworthy *et al.*, 2000). Several lines of evidence indicate that autoreactive T cells migrating from the periphery across the blood-brain barrier into the CNS may play a key role in the development of inflammatory response. Although functional studies revealed no alternations in NF-κB activity in T cells from patients with multiple sclerosis compared to healthy subjects, there is some evidence that NF-κB signaling pathways are involved in MS development (Flores *et al.*, 2003).

Concentrations of the activated p50 and p65 in the spinal cord of rats with experimental allergic encephalomyelitis (EAE) were found to correlate with the disease course — they were increased in the exacerbation period and decreased in the recovery phase (Pahan & Schmid, 2000). In turn, p50-deficient mice showed resistance to EAE, suggesting that this subunit is essential for disease development (Hilliard *et al.*, 1999).

In humans, a constant up-regulation of p65 was observed in oligodendrocytes and in macrophages from the active MS lesions, compared to the control white matter and silent MS plaques (Gveric *et al.*, 1998, Bonetti *et al.*, 1999). It is supposed that this constitutive p65 activation may facilitate a rapid response to the pathological stimuli in CNS and amplify the inflammatory reaction through the up-regulation of NF-κB-controlled genes encoding adhesion molecules and cytokines.

Type 1 diabetes mellitus

Type 1 diabetes mellitus (T1DM) is an autoimmune disease characterized by an inflammatory response resulting in a selective and progressive destruction of the insulin-secreting beta cells in the pancreas. Cumulative evidence suggests that destruction of beta cells is likely to be caused by a direct contact with the activated macrophages and with T lymphocytes, as well as with mediators secreted by these cells upon the activation of the NF-κB signaling pathway. Although basal NF-κB activation is required for the normal islet function, *in vitro* exposure of beta cells to the NF-κB triggering cytokine — IL-1β causes functional changes similar to those observed in pre-diabetic patients e.g. elevated proinsulin levels (Hostens *et al.*, 1999). In turn, activation of NF-κB by the synergic action of IL-1β and IFN-γ can induce beta cell apoptosis (Eizirik & Mandrup-Poulsen, 2001). NF-κB was also found to down-regulate the expression of some important transcription factors engaged in beta cell differentiation, proliferation and function, such as pancreas duodenum homeobox-1 (Kutlu *et al.*, 2003).

Several animal models have been used to study the role of NF-κB signaling in the development of T1DM *in vivo*. In one model, the NF-κB pathway was blocked during beta cell development by a transgenic expression of a degradation resist-

ant IκBα mutant able to prevent nuclear translocation of NF-κB dimers (Norlin *et al.*, 2005). The adult mice were hyperglycemic and had altered glucose-stimulated insulin secretion, revealing that blocking NF-κB during embryonic pancreas development results in the reduced expression of genes involved in insulin secretion as well as in a reduced number of endocrine cells in the adult pancreas. Nonobese diabetic mice that spontaneously develop diabetes are another commonly used animal model of T1DM. In autoreactive T cells derived from these mice, most authors describe an increased activation of NF-κB that also results in their higher survival rate (Bacher & Schmitz, 2004). However, one study reported decreased ability of NF-κB activation in T cells from nonobese diabetic mice. This defect was partly caused by a mutation in the promoter region of the gene encoding a large multifunctional peptidase 2 (LMP2) that resulted in a reduced protein level. Since LMP2 is one of the proteasome subunits involved in the degradation of IκBα, the overall effect of this mutation was a reduced NF-κB activity and an increase in the survival of a key population of highly activated and autoreactive T cells (Hayashi & Faustman, 1999).

Dendritic cells from nonobese diabetic mice also show elevated NF-κB activity due to IKK hyperactivation that results in an increased secretion of pro-inflammatory cytokines and in intense T cell activation, compared to DCs from control mice (Poligone *et al.*, 2002). Abnormalities of the NF-κB function were also observed in antigen presenting cells from patients with T1DM. In monocytes and monocyte-derived DCs isolated from T1DM patients, late LPS-mediated nuclear DNA binding by p50, p65, c-Rel and RelB was defective as compared with healthy subjects, and associated with impaired CD40 and MHC class I induction but with normal cytokine production (Mollah *et al.*, 2008).

Thyroid autoimmunity

The autoimmune thyroid diseases include Graves' disease (GD) and autoimmune hypothyroidism (AIH). Patients with GD present clinical manifestations of hyperthyroidism and in the majority of cases thyroid associated ophtalmopathy. A high proportion of GD patients have increased serum titers of thyroid autoantibodies including antibodies directed against the thyrotropin receptor which is thought to be the primary self-antigen. In contrast patients with AIH present with the clinical manifestations of an underactive thyroid gland and significant titers of thyroid peroxidase and/or thyroglobulin auto-antibodies (Simmonds & Gough, 2005).

Lymphocytes obtained from patients with GD and AIH produce high amounts of TNFα — a known trigger of the NF-κB signaling pathway, and the existence of TNFα receptors was demonstrated in human thyroid follicular cells, as well as on the fisher rat thyroid line-5 (FRTL-5) cells (Pang *et al.*, 1989). As was mentioned in the previous section, FRTL-5 cells were also found to express a functional TLR-3 that after viral stimulation can activate the NF-κB pathway. Immunohistochemical studies showed that TLR3 is also overexpressed in human thyreocytes surrounded by the immune cells in patients with AIH, suggesting that TLR3 overexpression can induce innate immune response in thyrocytes that may be important in the pathogenesis of AIH and in the immune cell infiltrates (Harii *et al.*, 2005).

In vitro studies performed on FRTL-5 cells revealed that thyrotropin as well as thyroid stimulating antibodies from patients with GD can modify activation of the NF-κB pathway by TNFα (Kikumori *et al.*, 2001). In the presence of thyrotropin or thyroid stimulating antibodies, both the p65/p50 heterodimer and p50/p50 homodimer were found to be activated after stimulation with TNFα, whereas in their absence — only the complex containing the p50 subunit. Activation of the p65/p50 heterodimer was associated with a higher expression of the IL-6 gene — a cytokine which plays a key role in promotion of B cell proliferation and differentiation and in T cell activation. Therefore NF-κB activation *via* thyrotropin or thyroid stimulating antibodies is believed to be an important mechanism in the development of the intra-thyroidal immune responses in patients with autoimmune thyroid diseases.

In turn, studies on animal models suggest that hyperthyroidism may influence humoral immunity *via* activation of the NF-κB signaling pathway. Antibody response in L-thyroxin treated rats to immunization with sheep red blood cells was found to be significantly higher compared to control animals, and correlated with p65 concentration in lymphocytes. Hence it seems that activation of the NF-κB signaling pathway in lymphocytes might be involved in hyperthyroid state induced potentation of humoral immune responses (Vinayagamoorthi *et al.*, 2005).

NF-κB also plays an important role in the pathogenesis of thyroid associated ophtalmopathy. Several pro-inflammatory cytokines (IL-1, TNFα and IFNγ) whose expression is regulated by the NF-κB signaling pathway, are known to play a central role in the autoimmune inflammation in orbital tissues. Moreover, apart from leukocyte activation, NF-κB is also involved in glycosaminoglycan production by orbital fibroblasts (Cao & Smith, 1999) that can be attenuated by the use of NF-κB inhibitors.

Systemic lupus erythematosus

Systemic lupus erythematosus (SLE) is a multisystem disease that is caused by antibody production and complement fixing immune complex deposition that results in tissue damage. As potentially many different antibodies can be produced in SLE patients, the different organ specific targets of these antibodies can cause a wide spectrum of clinical presentations, which are characterized by remissions and exacerbations.

Patients with SLE show constitutively high plasma levels of the B cell activating factor (BAFF) that is known to trigger a non-canonical NF-κB signaling pathway. High serum concentrations of BAFF were also found in murine models of lupus with an increase in serum BAFF concentrations over time paralleling worsening disease activity (Gross *et al.*, 2000). Interestingly, it was shown that some of BAFF-transgenic mice can display hyperglobulinemia, with circulating immune complexes and a positive rheumatoid factor, high titers of antibodies against single-stranded and double-stranded DNA, and immunoglobulin deposition in the kidneys — features characteristic for SLE-like diseases (Mackay *et al.*, 1999).

On the other hand, T cells isolated from patients with SLE were found to have a defect in T cell receptor-mediated activation of NF-κB signaling. This defect is probably caused by the lack of p65, whereas levels of p50 were similar to those observed in T cells from healthy donors (Wong *et al.*, 1999). Lack of the p65 subunit that possesses a C-terminal transcriptional activation domain should result in impaired ability to activate NF-κB target genes in lupus T cells. Indeed, functional studies revealed that T cells from SLE patients are defective in IL-2 synthesis after stimulation by mitogens (Murakawa *et al.*, 1985). Furthermore, it was shown that in the absence of p65 in lupus T cells, the remaining p50 can form homodimers, bind DNA and repress gene expression (Ballard *et al.*, 1992). In turn, cytosol concentration of c-Rel in T cells from patients with SLE was increased compared to cells derived from healthy individuals, however after stimulation, its nuclear import was defective and that was correlated with lower IL-2 synthesis (Burgos *et al.*, 2000). Impaired NF-κB signaling observed in SLE patients can also be partially explained by the decreased NF-κB ability of DNA binding (Oikonomidou *et al.*, 2006).

Inflammatory bowel diseases

Deregulated cytokine production and signaling mechanism in epithelial cells, mucosal lymphocytes and macrophages have been implicated in the pathogenesis of both Crohn's disease (CD) and ulcerative colitis (UC) — two main forms of inflammatory bowel diseases (IBD). There is no consensus whether IBD are typical autoimmune conditions since even though CD and UC resemble autoimmune diseases in many aspects, these so-called autoimmune features are non-specific and can be found in a wide variety of inflammatory diseases, infections, and other conditions. Moreover, despite a common basis, the two diseases have considerably different pathophysiologies. Studies on animal models suggest that the essential feature of CD inflammation is production of Th1-type cytokines, whereas UC is associated with atypical Th2 mediated responses. Given the implication of NF-κB in the regulation of both Th1 and Th2 responses, several studies were conducted to investigate a possible role of this signaling pathway in the pathogenesis of IBD (Fichtner-Feigl *et al.*, 2006). Immunochemistry of mucosa samples from patients with active CD showed increased concentration of activated p65 in epithelial cells and in lamina propria macrophages, compared to healthy controls (Schreiber *et al.*, 1998). Consistently, electromobility shift assays revealed increased constitutive NF-κB DNA binding in extracts from lamina propria biopsy samples and lamina propria mononuclear cells from patients with CD (Rogler *et al.*, 1998). High IKKβ activity that resulted in the recruitment of transcriptionally active p65 to the inducible nitric oxide synthase promoter was also observed in mucosa samples from patients with UC (Andresen *et al.*, 2005).

In IBD patients, the increased NF-κB expression in mucosal macrophages is accompanied by an increased capacity of these cells to produce and secrete TNF-α, IL-1, IL-6 and other cytokines that are directly involved in the mucosal tissue damage and responsible for further stimulation, activation and differentiation of lamina propria immune cells resulting in perpetuation of mucosal inflammation. The role of NF-κB in colon epithelium seems to be more complicated. On the one hand, IL-6 induced activation of NF-κB in colon epithelial cells is associated with expression of adhesion molecules important in the recruitment of neutrophils to the site of inflammation. On the other hand, cell-specific inhibition of NF-κB in the intestine *via* conditional ablation of NEMO, leads to the spontaneous development of severe intestinal inflammation in mice. NEMO-deficient intestinal epithelial cells revealed an increased rate of apoptosis and decreased production of antimicrobial peptide that results in an impaired integrity of the epithelial barrier and in an enhanced mucosal immune response, triggered by invading bacteria (Atreya *et al.*, 2008).

One of the animal models of intestinal inflammation used for testing novel therapeutic strategies is 2,4,6,-trinitrobenzene sulfonic acid (TNBS)-induced colitis that resembles human CD in terms of various

histological features including infiltration of colonic mucosa by neutrophils and macrophages and increased production of inflammatory mediators including Th1 profile of cytokines. Interfering with the NF-κB pathway either *via* administration of decoy oligodeoxynucleotides (ODN, described in the following sections) or by inhibition of the IKK activity in mice with TNBS-induced IBD leads to an abrogation of both acute and chronic intestinal inflammation (Neurath *et al.*, 1996; Fichtner-Feigl *et al.*, 2006; Ukil *et al.*, 2006). Decoy ODNs were also found to be effective in the treatment of Th2-mediated (oxazolone-induced) colitis in mice that reproduces many features of UC (Fichtner-Feigl *et al.*, 2006). In addition, this study demonstrated that inhibition of NF-κB may prevent the development of fibrosis that is a constant complication of long-standing IBD and can lead to some of the IBD associated symptoms such as loss of gut motility and obstruction. Moreover, treatment with the NF-κB decoy ODN was found to effectively minimize collagen deposition in the intestine, suggesting that this kind of therapy can also be effective in the attenuation of IBD-related complications.

Psoriasis

Psoriasis vulgaris is a chronic autoimmune inflammatory skin disease characterized by epidermal hyperproliferation with hyperkeratosis, intraepidermal leukocyte accumulation and cellular inflammation in the upper dermis. NF-κB has recently been found to play an important role in normal keratinocyte proliferation and function. Constitutive expression of p50, p65, RelB and c-Rel subunits was shown on both protein and mRNA levels in the cytosol of primary cultured keratinocytes as well as in immortalized keratinocyte lines (Takao *et al.*, 2003). In turn, immunohistochemical studies demonstrated relative absence of the nuclear active p65 in normal skin, its basal levels in non-lesional psoriatic skin and significant overexpression in the epidermis of psoriatic plaques (Lizzul *et al.*, 2005). Moreover, some alterations of the NF-κB-DNA binding in psoriatic lesions compared to non-lesional psoriatic skin were detected. Nuclear extracts from psoriatic lesions presented an increased NF-κB binding to the IL-8 (one of the causative cytokines involved in psoriasis development) promoter that was paralleled by the increased IL-8 mRNA and protein levels (Johansen *et al.*, 2005).

It has also been suggested that in the state of chronic inflammation in psoriasis, under the influence of many different cytokines, there is an imbalance between the anti-apoptotic role and cell-cycle inhibitory role of NF-κB that may lead to protection against cell death, that allows for the increased epidermal thickness and hyperproliferation seen in plaques (Johansen *et al.*, 2005). Interestingly, animals with disturbed NF-κB signaling present different skin defects: for instance IκBα knockout results in the hyperplasia of epidermal keratinocytes and dermal infiltration of lymphocytes, whereas p65 overexpression leads to the epidermal hypoplasia and growth inhibition (reviewed by Gerondakis *et al.*, 1999).

GENETIC DEFECTS OF THE NF-κB PATHWAY ASSOCIATED WITH AUTOIMMUNITY

Given the essential role of NF-κB signaling pathway in the development and progression of autoimmunity, genes encoding NF-κB related proteins constitute interesting candidates for association studies on genetic susceptibility towards autoimmune diseases. Indeed, genetic alterations leading to the deregulation of NF-κB signaling have been found in various autoimmune disorders both in human and in animal models.

A genome-wide screen in multiplex IBD pedigrees identified the region on chromosome 4q24, containing the *NFKB1* gene encoding the p105/p50 proteins, as a disease associated locus (Cho *et al.*, 1998). Subsequently, an association between the susceptibility to ulcerative colitis and a polymorphism located in the promoter region of the *NFKB1* gene (ATTG deletion) has been described (Karban *et al.*, 2004). This polymorphism was shown to decrease *NFKB1* transcriptional activity probably by interfering with binding sites for some crucial transcription factors. Given the important role of NF-κB signaling in the maintenance of integrity of the epithelial barrier, one can suspect that decreased transcriptional activity of *NFKB1* resulting in lower p50 concentration in epithelium may favor development of intestinal inflammation (Atreya *et al.*, 2008). Despite functional implications of this polymorphism, results of further studies could not unanimously confirm its association with UC (Borm *et al.*, 2005; Mirza *et al.*, 2005; Oliver *et al.*, 2005; Glas *et al.*, 2006). Moreover, case-control studies performed in different populations failed to replicate the association of the *NFKB1* polymorphism with some other autoimmune diseases (including T1DM, RA and SLE) (Orozco *et al.*, 2005; Kosoy & Concannon, 2005). However, a study performed in two different Polish populations revealed that the ATTG deletion in the promoter region of *NFKB1* can be associated with susceptibility to Graves' disease (Kurylowicz *et al.*, 2007). On the contrary, lack of the ATTG deletion has been recently associated with psoriasis in Chinese population (Li *et al.*, 2008), although an earlier study found no association of this polymorphism with development

of psoriatic arthritis in Caucasians (Butt *et al.*, 2005). Another variation — a highly polymorphic dinucleotide (CA) repeat microsatelite located in the regulatory region of the *NFKB1* gene was found to be associated with genetic predisposition to celiac disease in Spanish families (Rueda *et al.*, 2004) and to T1DM in UK (Hegazy *et al.*, 2001).The functional implications of this polymorphism remain unclear and another study did not confirm its association with T1DM in the Danish population (Gylvin *et al.*, 2002).

Genes encoding inhibitors of NF-κB also constitute interesting candidates for association studies on susceptibility to autoimmune diseases, since IκB mutations may result in the uncontrolled and persistent activation of NF-κB as well as in its constant inactivation. Recently, a single nucleotide polymorphism (SNP), a C/T substitution in position –826 in the promoter region of the *NFKBIA* gene (encoding IκBα) was found to be associated with development of RA and SLE in the Taiwanese population (Lin *et al.*, 2007a, Lin *et al.*, 2007b). *In silico* analysis revealed that this polymorphism is located near the putative binding site of the GATA-2 transcription factor and a functional study is being conducted to investigate the influence of this SNP on *NFKBIA* promoter activity (Lin *et al.*, 2007a). Another SNP, a G/A change in the 3'UTR region of *NFKBIA* (influencing transcript stability) was associated with development of Crohn's disease in German patients lacking other CD predisposing alleles (see below), but a subsequent study performed in Israel failed to confirm this association (Klein *et al.*, 2004; Leshinsky-Silver *et al.*, 2007).

Another NFκB related gene, encoding inhibitor of κB-like protein (IκBL) has been recently identified as a rheumatoid arthritis susceptibility locus. Moreover, a SNP located in the *IκBL* promoter region (an A/T substitution in position –62) was found to be associated with RA in Japanese (Okamoto *et al.*, 2003). This polymorphism disrupts a putative binding motive for the transcriptional repressor δEF1, involved in T cell development. It is supposed that a –62 A/T substitution may affect *IκBL* transcriptional activity and therefore — alter the immune response in the course of RA. This polymorphism was also associated with development of Graves' disease in the Polish population (Kurylowicz, unpublished). Interestingly, another polymorphism located in the *IκBL* exon 4 (C/T), leading to the amino acid substitution (C224R) was found to be associated with genetic predisposition to multiple sclerosis, especially in patients with a relapsing remitting course. This SNP disrupts in the predicted phosphorylation site and therefore can alter IκBL function (Miterski *et al.*, 2002).

Polymorphisms of two other genes related to the NF-κB signaling pathway, *NOD2* and *NOD1* encoding NRLs (receptors alternative to TLRs, expressed in gastric and intestinal cells, described in the previous sections) have been found to confer susceptibility to inflammatory bowel diseases. In the *NOD2* gene, three polymorphisms leading to protein changes at R702W, G908R and L1007finsC, were reported to be associated with development and clinical presentation of CD (Hugot *et al.*, 2001; Ogura *et al.*, 2001). The functional implications of these polymorphisms remain unclear, however some studies suggest that individuals possessing these variants have impaired NF-κB activation in response to LPS and peptidoglycans (Ogura *et al.*, 2001; Bonen *et al.*, 2003). On the other hand, mice with the above-mentioned mutations in the *nod2* gene produce higher levels of NF-κB and IL-1β when exposed to bacterial muramyl dipeptyde (Maeda *et al.*, 2005). Nevertheless, it is suggested that mutations associated with development of CD may result in the impaired ability of intestinal mucosa to control bacterial infection that in consequence can lead to the initiation of systemic responses and uncontrolled inflammation (Kucharzik *et al.*, 2006). Association of *NOD2* variants with CD in Caucasians has been widely replicated, however, studies on inheritance of the three risk alleles have demonstrated a remarkable heterogenity across ethnicities and populations with regional variation across Europe (reviewed by Cavanaugh, 2006). These polymorphisms seem to be specifically associated with CD, since other studies failed to confirm their contribution to other autoimmune diseases, including RA, psoriasis, SLE and T1DM (Pawlik *et al.*, 2007; Young *et al.*, 2003; Ferreiros-Vidal *et al.*, 2003; Ghandil *et al.*, 2005). Some studies indicated that the intronic $ND_1$32656 insertion/deletion polymorphism located in the *NOD1* gene can also be associated with CD, especially with the early onset form and with extraintestinal manifestations (McGovern *et al.*, 2005; Canto *et al.*, 2007), however these results have not been replicated in other populations (Tremelling *et al.*, 2006; Van Limbergen *et al.*, 2007).

Another genetic defect resulting in a decreased NF-κB activity was identified in patients with T1DM. This mutation affects a small ubiquitin related modifier 4 protein (SUMO-4) which takes part in IκBα ubiquitination and subsequent degradation (Fig. 1). A G163A SNP located in the *SUMO-4* gene, leading to the amino acid substitution (V55M) that alters gene transcriptional activity, was found to be associated with T1DM (Guo *et al.*, 2004; Bohren *et al.*, 2004). It is supposed that impaired degradation of IκBα can result in NF-κB cytoplasmic retention and partial elimination. Subsequent replication case-control and family-based studies have consistently confirmed this association in multiple Asian populations (reviewed by Wang & She, 2008). However, studies performed in Caucasians failed to confirm

the association of *SUMO-4* variants with susceptibility to T1DM (Smyth *et al.*, 2005; Sedimbi *et al.*, 2006). Genetic heterogeneity as well as gene–gene and gene–environment interactions have been suggested as possible reasons for these discrepancies observed among different populations. Indeed, a recently published study implies that *SUMO-4* polymorphism may contribute to the development of T1DM in Caucasians but only in association with high-risk HLA-DR3 and DR4 alleles (Sedimbi *et al.*, 2007).

The *SUMO-4* gene is located in the T1DM associated locus on the chromosome 6q25. This locus contains another NF-κB related gene — *TAB2* (known also as a mitogen-activating protein kinase 7 interacting protein 2). TAB2 is a protein required for the IL-1 and TNFα induced activation of the NF-κB signaling pathway, and a SNP (G/A substitution) in its gene was found to be associated with GD in UK Caucasians (Simmonds *et al.*, 2006).

Summarizing, to date only polymorphisms in the *NOD2* and *SUMO-4* genes can be treated as generally accepted susceptibility markers of autoimmune diseases: CD in Caucasians and T1DM in Asian populations, respectively. Studies regarding other NF-κB related genes either led to contradictory results and require a systematic meta-analysis, or are only preliminary reports that should be followed by large-scale replication studies. Investigated genetic variants in the NF-κB related genes and their association with development of autoimmune diseases are summarized in Table 1.

THERAPEUTIC PERSPECTIVES

The identification of NF-κB as a key player in development and progression of autoimmunity suggests that NF-κB-targeted therapeutics might be effective in the treatment of autoimmune diseases. Indeed, a number of novel therapeutic strategies that aim at the specific inhibition of key elements in the NF-κB pathway have been developed in the last few years.

Decoy oligodeoxynucleotides (ODN), proteasome inhibitors and peptides interfering with the nuclear translocation of NF-κB subunits have been shown to be effective in modulation of immune responses *in vitro*, as well as in treatment of autoimmune diseases on animal models.

Decoy ODNs, short double stranded DNAs containing a consensus sequence for the NF-κB binding sites, specifically block NF-κB binding to the target genes and are effective in the down-regulation of pro-inflammatory cytokine gene expression *in vitro* (Atreya *et al.*, 2008). Intra-articular administration of decoy ODN to rats with CIA resulted in decreased paw swelling, suppressed synovial production of IL-1β and TNFα, and in abrogated joint destruction (Tomita *et al.*, 2003). As it was mentioned above, NF-κB decoy ODNs were also efficient in suppression of IL-12, IFN-γ and IL-4 secretion in colonic lamina propria cells and in amelioration of experimental IBD symptoms (Fichtner-Feigl *et al.*, 2005). Unfortunately, ODNs are degradated rapidly *in vivo* and ODN-based therapy requires high doses and frequent, organ-specific administration that may hamper their use in the treatment of autoimmune diseases in humans (Isomura & Morita, 2006).

Proteasome inhibitors constitute a heterogenic group of compounds that can be generally categorized into two subgroups — natural products (e.g. a bacterial metabolite – lactacystin) and synthetic analogs (e.g. PS-341, PS-519). Whereas some of the proteasome inhibitors, like PS-341, have already been accepted for anti-cancer therapy in humans, to date their application in autoimmune diseases has been tested only on animal models. In rats with streptococcal cell wall induced polyarthritis (an animal model of RA), daily oral treatment with PS-341 alleviates the symptoms and attenuates the clinical progression of the disease that correlates with the lower serum levels of pro-inflammatory cytokines (Palombella *et al.*, 1998). In turn, mice with EAE treated with PS-519 exhibit significantly lower mean clinical disease scores and fewer relapses compared to the vehicle-treated controls which is associated with the profound reduction of T-cell infiltrations and demyelination in CNS (Vanderlugt *et al.*, 2000). PS-519 was also effective in reduction of inflammatory infiltrates in a SCID-hu xenogeneic psoriasis transplantation model, where lesional skin from patients with plaque-stage psoriasis was grafted onto mice lacking functional B and T cells (Zollner *et al.*, 2002).

Interference with the nuclear translocation of NF-κB is another potent strategy used for inhibition of NF-κB signaling. BMS-205820 is a cell-permeable peptide carrying two nuclear localization sequences, able to specifically block NF-κB nuclear localization resulting in the inhibition of cell surface protein expression, cytokine production, and T cell proliferation *in vitro*. *In vivo* BMS-205820 was found to be effective in the treatment of dextran sulfate sodium-induced model of IBD in mice (Fujihara *et al.*, 2000). Genetic constructs that overexpress IκB or express an engineered protein lacking sites for phosphorylation (an IκB super repressor) represent another approach to inhibition of NF-κB nuclear translocation that was effective in the treatment of animal models of RA and T1DM, as described previously (Miagkov *et al.*, 1998, Eldor *et al.*, 2005).

Given the crucial role of IKKs in NF-κB activation and nuclear translocation, these proteins also constitute an appealing target for therapeutic intervention. As it was previously described, intra-articu-

Table 1. Association of polymorphisms in NF-κB related genes with susceptibility to autoimmune diseases in different population.

Gene (protein)	Polymorphism	Disease	Population Association	Lack of association	References
NFKB1 (p105/p50)	promoter –94 ATTG ins/del	Ulcerative colitis	US Caucasians		Karban *et al.*, 2004
			Israeli		Karban *et al.*, 2004
			Dutch Caucasians		Borm *et al.*, 2005
				Spanish Caucasians	Oliver *et al.*, 2005
				German Caucasians	Glas *et al.*, 2006
		Crohn's disease		Dutch Caucasians	Borm *et al.*, 2005
				German Caucasians	Glas *et al.*, 2006
		Type 1 diabetes mellitus		US and UK Caucasians	Kosoy & Concannon, 2005
		Rheumatoid arthritis		Spanish Caucasians	Orozco *et al.*, 2005
		Systemic lupus erythematosus		Spanish Caucasians	Orozco *et al.*, 2005
		Graves' disease	Polish Caucasians		Kurylowicz *et al.*, 2007
		Psoriasis	Chinese		Li *et al.*, 2008
				Canadian Caucasians	Butt *et al.*, 2005
	(CA)n dinucleotide repeat	Type 1 diabetes mellitus	UK Caucasians		Hegazy *et al.*, 2001
				Danish Caucasians	Gylvin *et al.*, 2002
		Celiac disease		Spanish Caucasians	Rueda *et al.*, 2004
NFKBIA (IκBα)	promoter –826 C/T	Rheumatoid arthritis	Taiwanese		Lin *et al.*, 2007a
		Systemic lupus erythematosus	Taiwanese		Lin *et al.*, 2007b
	3′UTR G/A	Crohn's disease	German Caucasians		Klein *et al.*, 2004
				Israeli	Leshinsky-Silver *et al.*, 2007
IκBL (IκBL)	promoter –62 A/T	Rheumatoid arthritis	Japanese		Okamoto *et al.*, 2003
		Graves' disease	Polish Caucasians		Kurylowicz, unpublished
	exon4 C/T (C224R)	Multiple sclerosis	German Caucasians		Miterski *et al.*, 2002
NOD2 (NOD1)	R702W, G908R, L1007finsC	Crohn's disease*	US Caucasians		Ogura *et al.*, 2001
			French Caucasians		Hugot *et al.*, 2001
		Rheumatoid arthritis		Polish Caucasians	Pawlik *et al.*, 2007
		Psoriasis		UK Caucasians	Young *et al.*, 2003
		Type 1 diabetes mellitus		French Caucasians	Ghandil *et al.*, 2005
		Systemic lupus erythematosus		Spanish Caucasians	Ferreiros-Vidal *et al.*, 2003
NOD1 (NOD1)	$ND_1$32656 ins/del	Crohn's disease	UK Caucasians		McGovern *et al.*, 2005
			Spanish Caucasians		Canto *et al.*, 2007
				UK Caucasians	Tremelling *et al.*, 2006
				UK Caucasians	Van Limbergen *et al.*, 2007
				Swedish Caucasians	
SUMO-4 (SUMO-4)	A163G	Type 1 diabetes mellitus**	US Caucasians		Bohren *et al.*, 2004
			US Caucasians		Guo *et al.*, 2004
			Spanish Caucasians		
			Chinese		
			Taiwanese		
			Korean		
			Swedish Caucasians		Sedimbi *et al.*, 2007
				Lativian Caucasians	Sedimbi *et al.*, 2006
				UK Caucasians	Smyth *et al.*, 2005
TAB2 (TAB2)	G/A (001Msp)	Graves' disease	UK Caucasians		Simmonds *et al.*, 2006

*Association of *NOD2* variants with Crohn's disease in Caucasians was widely replicated (for review see Cavanaugh *et al.*, 2006). **Association of *SUMO-4* variants with type 1 diabetes mellitus was confirmed in multiple Asian populations (for review see Wang & She, 2008).

lar delivery of a dominant negative IKKβ adenovirus construct as well as administration of highly specific IKKβ inhibitor — a NEMO-binding domain peptide, were able to reduce autoimmune arthritis activity in animal models (Tak *et al.*, 2001; Tas *et al.*, 2006). Small molecule inhibitors constitute the next group of IKK-targeted compounds intensively studied for their efficacy in autoimmune disease treatment. A representative of this group, SC-514 is able to inhibit IκB phosphorylation in RA synovial fibroblasts *in vitro* (Kishore *et al.*, 2003) whereas administration of another small molecule IKKβ inhibitor — BMS-345541 — ameliorates symptoms of CIA and attenuates colitis symptoms in mouse models (McIntyre *et al.*, 2003; MacMaster *et al.*, 2003). Novel, intensively developing NF-κB targeted strategies comprise RNA interference with the use of anti-NF-κB RNA aptamers or small interfering RNAs (Chan *et al.*, 2006).

Apart from the novel, specific NF-κB-directed therapies, a variety of drugs used in the conventional treatment of autoimmune diseases have effects on NF-κB activity. Glucocorticoids are an example of commonly used therapeutics that can modulate the NF-κB pathway through several proposed mechanisms. Glucocorticoids were found to induce expression of the *NFKBIA* gene, causing an increased cytosolic retention of NF-κB. Next, they may also interfere with NF-κB DNA-binding activity *via* direct interaction between the glucocorticoid receptor and components of the NF-κB binding sites in various gene promoters. Moreover, in certain types of cells, the glucocorticoid receptor can also directly interact with the NF-κB p65 subunit and interfere with its transactivation potential. Finally, competition may occur between the glucocorticoid receptor and NF-κB, limiting amounts of the transcription coactivators (reviewed by De Bosscher *et al.*, 2003).

Cyclosporin A and tacrolimus (FK506), two immunosuppressive agents administered in a variety of disease conditions such as clinical post-organ transplantation and autoimmune diseases, also exert their function by the inhibition of the NF-κB pathway. Cyclosporin A was found to act as a noncompetitive proteasome inhibitor *in vitro* and to suppress LPS-induced IκB degradation and p105 processing *in vivo* (Meyer *et al.*, 1997). In turn, tacrolimus blocks nuclear translocation of the c-Rel subunit in B and T cells, leading to the decreased expression of IL-2 and its receptor (Venkataraman *et al.*, 1995). Interfering with NF-κB signaling was also found to be an important mechanism of cyclosporin A and tacrolimus mediated down-regulation of LPS-induced IL-12 synthesis in monocytes (Ma *et al.*, 2007).

Non-steroidal anti-inflammatory drugs used in the treatment of RA, such as aspirin, phenylbutazone, naproxen, diclofenac, ibuprofen or sulindac, were found (with differing efficacy) to prevent activation of the NF-κB pathway by the competitive inhibition of the ATP-binding site of IKK thereby imparing IκB phosphorylation (Takada *et al.*, 2004). Sulfasalazine, commonly used in the treatment of IBD and RA, after transformation to its active derivate — 5-aminosalicylic acid, was also shown to suppress IκB phosphorylation *via* direct inhibition of IKKα and IKKβ (Weber *et al.*, 2000). However, this action is not specific and requires relatively high concentrations to achieve effective NF-κB inhibition.

FINAL CONCLUSIONS

Although autoimmune diseases, which collectively affect up to 10% of the general population, are relatively common, the cascade of events that lead to their development is still largely unknown. NF-κB, as a key transcription factor involved in the regulation of immune responses and apoptosis, appears to be a good candidate for studies on mechanisms of self-tolerance failure. Indeed, recent years have brought a lot of data suggesting that the NF-κB signaling pathway is frequently affected and altered in several autoimmune diseases. Polymorphisms in genes encoding NF-κB related proteins NOD2 and SUMO-4 can be considered as susceptibility markers of two autoimmune diseases: Crohn's disease in Caucasians and type 1 diabetes mellitus in Asian populations, respectively. Even though sometimes it is not clear whether aberrant NF-κB activity is a causative mechanism or just a consequence of ongoing inflammation, inhibition of NF-κB signaling was found to be effective in the treatment of autoimmune disorders. Since NF-κB is essential for cellular homeostasis, of some concern is the lack of specifity of NF-κB blockade which may interfere with normal cellular functions and lead to toxicity. However, by targeting specific NF-κB subunits, IκB proteins or IKK that present tissue specificity, a therapeutic efficacy and minimalization of systemic toxicity may be obtained. Therefore, identification of individual NF-κB components as key to a particular disease and development of inhibitory compounds able to exert tissue-specific activity will be a promising and challenging task for the future.

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