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Review

Immunopharmacology: anti-inflammatory therapy targeting transcription factors

Josef Pfeilschifter *, Heiko Mühl

Zentrum der Pharmakologie, Klinikum der Johann Wolfgang Goethe-Universität, Theodor-Stern-Kai 7, D-60590 Frankfort on the Main, Germany

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Abstract

Immunopharmacology is one of the most dynamic areas in pharmacology encompassing classical immunosuppressive drugs which reveal completely new clues concerning their mode of action as well as novel molecular biology approaches for treating inflammatory and autoimmune diseases, infections and cancer. This article focuses on transcription factors that regulate cell activities involved in immune and inflammatory cell responses and how traditional anti-inflammatory compounds such as glucocorticoids, cyclosporins, tacrolismus and salicylates interfere with the activation cascades triggering the transcription factors. Moreover, promising new initiatives for selective therapeutics including recombinant anti-inflammatory cytokines and proinflammatory cytokine antagonists, and gene therapy will be presented.

Keywords: Inflammation; Immunopharmacology; Gene expression; Transcription factor; Glucocorticoid; Cyclosporin A; Tacrolismus; Salicylate; Aspirin; Recombinant cytokine; Gene therapy

1. Introduction

During the last decade, immunopharmacology has emerged as one of the most dynamic areas of pharmacological research. Whereas until most recently, research largely depended on chance observations leading to the discovery of small active compounds, today immunopharmacology changes its scope towards the rational use of endogenous modulators targeting cell growth, infections, and immune responses. This change towards, in this sense, a biologic therapy is intimately linked to recent rapid progress in the field of molecular biology and biotechnology (Ballow and Nelson, 1997). Presently, therapeutic strategies are under development that will interfere with disease progression by use of recombinant proteins (e.g., interleukin-1 receptor antagonist, interleukin-18-binding protein, interleukin-10) or by use of gene therapy approaches. Somatic gene therapy may include specific overexpression of certain genes (e.g., cytokines, anti-oncogenes) as well as expression of antisense molecules or ribozymes. Conventional drug therapy uses potent and successful agents such as glucocorticoids and salicylates, as well as immunosuppressants like cyclosporin A or anti-metabolites like methotrexate and other chemotherapeutic agents. It is tempting to speculate that in future new therapeutical approaches based on recombinant gene technology will complement conventional treatments of human disorders like cancer, AIDS, or rheumatoid arthritis.

Furthermore, the instrument of molecular biology will provide scientists with more detailed knowledge regarding mechanisms of action of conventional drugs. Novel information might enable researchers to improve therapies based on these drugs.

During the last decade, there has been tremendous progress in the field of cytokine biology. A central role for cytokines such as interleukin-1, interleukin-10 and tumor necrosis factor α particularly in the pathogenesis of inflammatory and autoimmune diseases has been demonstrated consistently in numerous studies (Dinarello, 1996; Lalani et al., 1997; Cope, 1998; van der Meer et al., 1998). These findings have opened the new field of anti-cytokine therapy for the treatment of autoimmune as well as acute and chronic inflammatory diseases (Eigler et al., 1997; Firestein and Zvaifler, 1997). In the present review, we will focus on new aspects in the treatment of this group of disorders. We will review recent progress in our understanding of molecular mechanisms which mediate the ef-

* Corresponding author. Tel.: +49-69-6301-6951; fax: +49-69-6301-7942; E-mail: pfeilschifter@em.uni-frankfurt.de

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fects of drugs like dexamethasone, cyclosporin A, or salicylates and we will discuss the novel approaches of cytokine therapy and gene therapy in the context of inflammatory and autoimmune diseases.

2. Transcription factor-targeting therapy

A critical determination of immunological phenomena is the expression of cytokines and other protein factors by immune cells. In turn, these factors initiate an effective response of the organism. The ability to regulate gene expression in response to changes in the environment is a prerequisite for an adequate adaptation of immune cells and other cell types. Changes in gene expression are regulated by the activity of transcription factors which in turn control RNA polymerase II activity. Transcription factors bind to specific DNA sequences and trigger gene transcription by directly interacting with the basal transcriptional machinery. In addition to classical pharmacological approaches which aim at direct inhibition of protein products encoded by genes like enzymes, transporters or channels, novel methods of blocking gene expression may lead to new therapies for human diseases (Gottesfeld et al., 1997). Antisense oligodesoxynucleotides show great promise as drugs for the specific alteration of gene expression (Stein, 1996) and the rapid progress in the field of transcriptional regulation has opened up the possibility of the targeting of transcription factors that are involved in human diseases (Peterson and Baichwal, 1993; Papavassiliou, 1998). Targeting gene-specific factors has the advantage of modulating the expression of only a limited number of genes and dependent on whether the targeted factor is a transcriptional activator or repressor, gene expression will be reduced or increased, respectively. There are indeed examples of important classes of drugs that modulate transcription factors and subsequent expression of target genes. These therapeutics include steroid hormones and their antagonists, cyclosporin A and salicylates, to name just a few examples. Obviously, transcription factor-targeting drugs have the potential to become block-buster therapeutics.

3. Glucocorticoids

Glucocorticoids are among the most potent and widely used anti-inflammatory and immune-modulatory drugs. Administration of corticosteroids is standard therapy for the management of diseases such as arthritis, asthma, inflammatory bowel disease, systemic lupus erythematosus and prevention of transplant rejection. The mode of action of this important class of drugs comprises a variety of mechanisms including transcriptional, post-transcriptional, translational and post-translational actions. Glucocorticoids inhibit the expression of proinflammatory cytokines such as interleukin-1, interleukin-2, interleukin-6, interleukin-8, tumor necrosis factor α and interferon-γ and simultaneously enhance the expression of anti-inflammatory interleukin-1 receptor antagonist. Glucocorticoids also alter recruitment and activation of professional inflammatory cells such as monocytes/macrophages, eosinophils or lymphocytes. Inhibition of adhesion molecules on endothelial cells and inhibition of chemokine expression also impairs the recruitment of neutrophils to the site of inflammation (Barnes and Adcock, 1993; Wilckens and de Rijk, 1997). In addition, glucocorticoids inhibit the generation of inflammatory mediators like prostaglandins, thromboxanes, leukotrienes and nitric oxide by suppression of gene expression of cytosolic (Schalkwijk et al., 1993) and secretory phospholipase A_2 (Schalkwijk et al., 1991), cyclooxygenase-2 (DeWitt and Meade, 1993) as well as of inducible NO-synthase (Pfeilschifter and Schwarzenbach, 1990; Kunz et al., 1996). The gene regulatory aspects of glucocorticoids are generally thought to be mediated by its intracellular receptor, the glucocorticoid receptor, which usually resides in the cytoplasm of cells complexed to chaperones like heat shock protein 90 (Dalman et al., 1991) and one p59 immunophilin molecule. Upon binding of a specific steroid, the glucocorticoid receptor · heat shock protein 90 · p59 immunophilin complex dissociates and the free glucocorticoid receptor and its steroid ligand are imported into the nucleus where the glucocorticoid receptor dimerizes and binds to specific DNA sequences termed glucocorticoid response elements in the promoter regions of target genes (Zilliacus et al., 1995). Glucocorticoid response elements comprise palindromic DNA motifs of 15 base pairs in length and stimulate target expression. The negative regulatory glucocorticoid response elements which mediate repression of target genes display a more variable DNA sequence. By subtraction cloning studies, RNA transcript complexity studies and two-dimensional protein gel analysis the number of glucocorticoid-regulated genes has been estimated at 10 to 100 (Briehl et al., 1990; Owens et al., 1991). Although the glucocorticoid receptor is widely expressed glucocorticoids regulate transcription in vivo in both a promoter- and tissue-specific manner. Both the DNA sequence at the promoter and other transcriptional factors bound to the promoter define the specific requirements for transcriptional alterations triggered by the glucocorticoid receptor (Guido et al., 1996). Several natural or synthetic ligands can impinge glucocorticoid receptor action and are used to this end under experimental and clinical conditions.

A further level of regulation affected by glucocorticoid receptor is the modulation of other transcription factors which occurs independent of DNA binding through direct protein–protein interactions (McEwan et al., 1997). In this way, the glucocorticoid receptor traps transcription factors such as activator protein-1, nuclear factor κB, GATA-1 (guanosine–adenosine–thymidine–adenosine) and cyclic AMP-responsive element-binding protein and prevents transactivation by these factors resulting in repression of

gene expression (Barnes and Adcock, 1993; McEwan et al., 1997; Reichardt and Schütz, 1998). This mechanism was first described for activator protein-1 (Jonat et al., 1990; Schüle et al., 1990; Yang Yen et al., 1990) and found to be independent of protein synthesis, strongly suggesting that glucocorticoid receptor interferes with activator protein-1 activity by direct protein–protein interactions. There is evidence that the glucocorticoid receptor binds to c-Fos and c-Jun subunits of activator protein-1 (Cato and Wade, 1996). The integrity of the DNA-binding domain of the glucocorticoid receptor appears to be essential for the repressive activity (Heck, 1994). Alternatively, competition for a limiting cofactor such as the binding protein for cyclic AMP-responsive element-binding protein/p300 has been proposed (Kamei et al., 1996). Moreover, Caelles et al. (1997) suggested that inhibition of c-Jun phosphorylation which is mandatory for activator protein-1 activation is responsible for glucocorticoid-induced gene repression. This type of cross-talk between activator protein-1 and glucocorticoid receptor is responsible for repression of genes like the one coding for the metalloproteinases of the collagenase family which have a critical activator protein-1 site in their promoters (Pfahl, 1993; Cato and Wade, 1996). Another prototypical transcription factor that can be affected by glucocorticoids and mediate effects on genes whose promoters lack positive or negative glucocorticoid response elements is nuclear factor κB. This transcription factor is a pleiotropic regulator of many inducible genes involved in immune and inflammatory reactions including those for cytokines, chemokines, adhesion molecules, acute phase proteins and enzymes that generate inflammatory mediators (Baeuerle, 1998; May and Gosh, 1998). Nuclear factor κB is a preformed-transcription factor that is rendered inactive in the cytosol by an inhibitory anchor protein called inhibitor of κB. In response to activating stimuli, the inhibitor of κB is phosphorylated and subsequently degraded. This unmasks the nuclear localization sequence of nuclear factor κB and triggers the nuclear import of the transcription factor which binds to its recognition DNA motif and participates in the activation of gene transcription. Many of the target genes regulated by nuclear factor κB are repressed by glucocorticoids including those for interleukin-2, interleukin-6, interleukin-8, inducible NO-synthase or phospholipase A_2. There are two ways by which glucocorticoids can interfere with nuclear factor κB-induced gene transcription. The first and most important way is by direct protein–protein interaction. In the cell nucleus, the glucocorticoid–glucocorticoid receptor complex interacts with the active transactivating p65 (RelA) subunit of nuclear factor κB and prevents nuclear factor κB binding to the DNA recognition motif in the promoter regions of target genes (Ray and Prefontaine, 1994; Caldenhoven et al., 1995; Brostjan et al., 1996). The N-terminal Rel-homology domain of p65 and the zinc-finger structure of the glucocorticoid receptor DNA-binding motif are required for this interaction (Liden et al., 1997; Wissink et al., 1997). The second way that applies for only a restricted number of cell types is the glucocorticoid-induced expression of the inhibitor of κB that subsequently traps the active nuclear factor κB and thus turns off nuclear factor κB-driven gene transcription (Auphan et al., 1995; Scheinman et al., 1995). The in vivo relevance of DNA-binding independent activities of the glucocorticoid receptor was most elegantly demonstrated by introduction of a point mutation into the glucocorticoid receptor which impaired dimerization (glucocorticoid receptor^{dim}) and therefore glucocorticoid response element-dependent transactivation while leaving interaction with other transcription factors intact (Reichardt and Schütz, 1998). In contrast to homozygous mice with a disrupted glucocorticoid receptor gene which die shortly after birth due to severe lung defects, the mutant mice (glucocorticoid receptor^{dim}) were viable displaying no histological abnormalities in the lung and adrenals (Reichardt and Schütz, 1998). It is tempting to speculate that ligands for the glucocorticoid receptor that only affect DNA-binding-independent functions of the glucocorticoid receptor will provide an improved spectrum of anti-inflammatory and immunosuppressive activities and less side-effects.

So far, only a few reports demonstrated that post-transcriptional mechanisms including protein translation and protein secretion may also be targets of the anti-inflammatory actions of glucocorticoids. Recent investigations have shown that dexamethasone inhibits inducible NO-synthase expression in interleukin-1β-stimulated renal mesangial cells (Kunz et al., 1996) and in interferon-γ-stimulated murine macrophages (Walker et al., 1997a) by prominent post-transcriptional actions. The steroid drastically enhanced inducible NO-synthase degradation by a calpain I-mediated mechanism (Walker et al., 1997a). Two structural determinants are involved in cleavage by calpain I, a conformational determinant and the calmodulin-binding domain (Walker, Pfeilschifter, Kunz, unpublished observations).

4. Cyclosporin and tacrolismus

The immunosuppressants cyclosporin and tacrolismus (FK506), two chemically different natural products, prevent graft rejection after organ transplantation. These drugs bind to their specific cytosolic receptors, cyclophilin and FK-binding protein, respectively, and as receptor–drug complexes, they highly selectively inhibit calcineurin, a calcium and calmodulin-dependent protein phosphatase also denoted as protein phosphatase 2B. Calcineurin is required for the dephosphorylation of the cytosolic form of the transcription factor family nuclear factor of activated T-cells (Schreiber and Crabtree, 1992; Liu, 1993). Dephosphorylation of nuclear factor of activated T-cells causes the exposure of two nuclear localization sequences and

triggers the subsequent import of the transcription factor into the nucleus where it cooperatively binds to specific DNA recognition motifs together with activator protein-1, GATA-4 and others that were originally designated nuclear form of nuclear factor of activated T-cells (Liu, 1993). By inhibiting nuclear factor of activated T-cells' activation cyclosporin and tacrolismus block T-cell function and the production of critical cytokines such as interleukin-2, tumor necrosis factor α and granulocyte-macrophage-colony-stimulating-factor in the course of alloantigen stimulation. Moreover, calcineurin has also been reported to stimulate nuclear factor κB by enhancing inactivation of its inhibitor of κB (Frantz et al., 1994) and consequently, cyclosporin A and tacrolismus may inhibit T-cell proliferation partly also because of inhibition of nuclear factor κB. Further on, inhibition of nuclear factor κB by cyclosporin and tacrolismus may furnish these drug with potent anti-inflammatory potentials. Indeed, cyclosporin and its derivatives were found to inhibit interleukin-1β-induction of inducible NO-synthase (Mühl et al., 1993; Kunz et al., 1995) and secretory phospholipase A_2 (Walker et al., 1997b) by reducing DNA-binding of nuclear factor κB in renal mesangial cells (Kunz et al., 1995; Walker et al., 1997b). The mode of action of cyclosporin and tacrolismus to inhibit nuclear factor κB is not yet known. Whether the drugs interfere with the phosphorylation of inhibitor of κB (Frantz et al., 1994) or its proteolytic degradation in order to stabilize inhibitor of κB (Marienfeld et al., 1997) remains to be elucidated. It is, however, noteworthy that cyclosporin A acts as an uncompetitive inhibitor of proteasome activity in vitro and inhibited endotoxin-induced inhibitor of κB degradation in vivo (Meyer et al., 1997). Recently, it has been reported that cyclosporin activates the transforming growth factor β gene in a variety of cell types and thus induces cancer progression (Hojo et al., 1999). As transforming growth factor β also potently blocks expression of inflammatory mediators like inducible NO-synthase (Pfeilschifter and Vosbeck, 1991) and secretory phospholipase A_2 (Pfeilschifter et al., 1990; Mühl et al., 1992; Schalkwijk et al., 1992), this may partially contribute to cyclosporin-induced anti-inflammatory activity.

5. Aspirin and salicylates

Aspirin and its derivatives are the most widely used drugs on a worldwide basis. At low therapeutic doses, aspirin irreversibly inhibits the activity of cyclooxygenases and the subsequent formation of prostaglandins. At higher therapeutic doses, aspirin has additional anti-inflammatory effects that are not related to inhibition of prostaglandin synthesis. Kopp and Sankar (1994) have reported that sodium salicylate and aspirin inhibit nuclear factor κB binding to its DNA recognition motifs. Very recently, Yin et al. (1998) reported that high concentrations of aspirin (IC_{50} approx. 50 μM) and sodium salicylate inhibit the enzyme inhibitor of κB kinase-β which may explain the clinical efficacy of high-dose aspirin. Phosphorylation of inhibitor of κB by inhibitor of κB kinase-β is required for the degradation of inhibitor of κB and the release of nuclear factor κB which subsequently is imported into the nucleus and triggers gene activation (Baeuerle, 1998; May and Gosh, 1998). The mechanism of aspirin and sodium salicylate inhibition of inhibitor of κB kinase-β is due to competition with ATP for binding to the catalytic domain of the kinase. Several other kinases, including the homologous and functionally related inhibitor of κB kinase-α, are not affected by aspirin and sodium salicylate (Yin et al., 1998). High concentrations of aspirin have also been reported to block the activity of the Jun-N-terminal kinases (Schwenger et al., 1997) which mediates phosphorylation and activation of the transcription factor c-Jun. An important consequence from this work is that inhibitor of κB kinase-β is an attractive molecular target for novel anti-inflammatory drugs with hopefully a better separation of undesirable from the desirable effects.

6. Anti-cytokine strategies using recombinant cytokines or gene therapy: novel therapeutic approaches for the treatment of autoimmune and inflammatory diseases as illustrated for the systemic inflammatory response syndrome and rheumatoid arthritis

Systemic inflammatory response syndrome is defined as systemic inflammation induced by infection which is in the majority of cases (about 50%) associated with gram-negative bacteria manifestation, and constitutes one major cause of death in modern intensive care units. Multi-organ failure and refractory hypotension/septic shock are the main causes of death related to systemic inflammatory response syndrome with a mortality rate ranging well above 60% (Brun-Buisson et al., 1995; Bates et al., 1997). From the molecular point of view, the fatal chain of events in systemic inflammatory response syndrome is initiated by lipopolysaccharide/endotoxin-driven release of inflammatory mediators such as interleukin-1, tumor necrosis factor α, interleukin-6, chemokines, platelet-activating factor, eicosanoids, and nitric oxide (Dinarello et al., 1993; Tetta et al., 1997; Cain et al., 1998; Wolkow, 1998).

Since recombinant biotechnology has become available in the early 1990s, clinical trials have been conducted in order to specifically block the effects of the two major inflammatory cytokines interleukin-1 and tumor necrosis factor α. The interleukin-1 receptor antagonist which binds to interleukin-1 type I receptor but lacks agonistic activity, has become a valuable tool for assessing the role of interleukin-1 in pathophysiology (Arend et al., 1998). Since interleukin-1 receptor antagonist can be given intravenously to healthy humans without causing any effects (Granowitz et al., 1992), the molecule became the focus of

clinical trials aiming at blockage of interleukin-1 activity in disease. However, a phase III trial in sepsis patients turned out to be inconclusive in that only a non-significant 9% reduction of 28–30 day all-cause mortality was noted (Opal et al., 1997). Recombinant soluble tumor necrosis factor receptors (p55 as well as p75) fused to the F_c portion of immunoglobulin G have been used for clinical trials investigating the outcome in septic patients during tumor necrosis factor α blockage. As was the case for interleukin-1 receptor antagonist, the results obtained were either inconclusive, or showed a modest or rather complex effect of these molecules. The soluble tumor necrosis factor receptor p75 given in a phase II trial did not reduce mortality in septic shock at all. In contrast, there was significant increase in mortality at the highest concentration (Fisher et al., 1996). The soluble tumor necrosis factor receptor p55 in a phase III trial did not significantly change the 28-day all-cause mortality in the whole group of patients (refractory shock patients and severe sepsis patients). However, in the subgroup with severe sepsis and early septic shock, there was a significant 36% reduction of mortality after soluble tumor necrosis factor receptor p55-immunoglobulin G given in the highest concentration (Abraham et al., 1997). Overall, these results indicate that inhibition of both, interleukin-1 and tumor necrosis factor α at the same time, might be necessary in order to obtain more striking effects on the course of sepsis.

Several studies characterize interleukin-10 as a macrophage deactivating cytokine (Bogdan et al., 1991; Berkman et al., 1995). Thus, interleukin-10 appears to be a candidate for cytokine treatment in systemic inflammatory response syndrome. In a phase I trial, interleukin-10 was given intravenously to healthy donors for various time periods. Thereafter, blood was drawn and endotoxin-stimulated production of interleukin-1β, tumor necrosis factor α, interleukin-8, interleukin-1 receptor antagonist, and soluble tumor necrosis factor p55 was evaluated ex vivo in whole blood cultures or peripheral blood monocytic cells. Interleukin-10 application significantly decreased release of proinflammatory cytokines induced by endotoxin, but did not reduce levels of the respective cytokine antagonists interleukin-1 receptor antagonist and soluble tumor necrosis factor receptor p55 (Fuchs et al., 1996). Other promising candidates for this type of cytokine therapy are interleukin-4 and interleukin-13, which like interleukin-10 belong to the group of T-helper-2 cytokines. Both cytokines promote the synthesis of interleukin-1 receptor antagonist but reduce production of interleukin-1 in endotoxin-stimulated human monocytes or peripheral blood monocytic cells. Thus, interleukin-4 and interleukin-13 can be regarded as anti-inflammatory (Vannier et al., 1992, 1996). Future clinical trials should provide more information concerning a potential clinical benefit of these T-helper-2 cytokines in sepsis.

The newly characterized cytokine interleukin-18 (Okamura et al., 1995; Dinarello et al., 1998) has been implicated in the pathophysiology of endotoxic shock. Recently, an interleukin-18 antagonist called interleukin-18-binding protein has been identified. Interleukin-18-binding protein significantly downregulated production of interferon-γ in response to endotoxin in mice in vivo (Novick et al., 1999). Since interferon-γ is a major mediator of endotoxin-induced lethality in endotoxemia (Doherty et al., 1992) and interleukin-18 induces interleukin-1, tumor necrosis factor α, as well as chemokines in human peripheral blood monocytic cells (Puren et al., 1998), interleukin-18-binding protein is definitely another promising cytokine antagonist for future trials in the field of anti-cytokine-therapy in systemic inflammatory response syndrome.

Rheumatoid arthritis is regarded as an autoimmune disease which is characterized by persistent immune activation and excessive formation of proinflammatory cytokines. Cytokine-driven chronic inflammation and accompanied tissue destruction results in clinical manifestation of disease and may finally progress to loss of joint function (Isomaki and Punnonen, 1997). For the treatment of rheumatoid arthritis using anti-cytokine approaches, the same set of molecules are under focus as in the studies dealing with systemic inflammatory response syndrome. Neutralizing the activities of interleukin-1 or tumor necrosis factor α in phase II or phase III clinical trials revealed the potentially beneficial effect of the anti-cytokine strategy in rheumatoid arthritis. Treatment of patients with interleukin-1 receptor antagonist resulted in significant improvement of clinical symptoms like swollen or tender joints, stiffness, and pain (Bresnihan et al., 1998). In analogy, blockage of tumor necrosis factor α by soluble tumor necrosis factor receptor p75-immunoglobulin G led to significant reduction of disease activity in patients. There were no dose-limiting toxic effects noted in the patients receiving soluble tumor necrosis factor receptor p75-immunoglobulin G (Moreland et al., 1997). In comparison to interleukin-1 receptor antagonist and soluble tumor necrosis factor receptor p75, much more limited information is available regarding the therapeutic potential of interleukin-4 and interleukin-10 in the human system. However, in ex vivo studies using mononuclear cells from rheumatoid arthritis patients, interleukin-4 and interleukin-10 reduced production of interleukin-1 and tumor necrosis factor α induced by bacterial antigen. Simultaneously, interleukin-10 increased release of prostaglandins from cartilage (van Roon et al., 1996), which may mediate an anti-inflammatory effect (Knudsen et al., 1986). Clinical trials will provide more facts regarding the efficacy particularly of interleukin-10 in the treatment of rheumatoid arthritis.

6.1. Gene therapy

The most far-reaching definition of somatic gene therapy is introduction of foreign nucleic acid into somatic cells of an organism with the objective to achieve amelio-

ration of disease. Gene therapy may aim at replacement of a missing or mutated gene, or either at enhancement or blockage of normal gene function. Gene therapy approaches can be divided into protocols in which the somatic cells are manipulated ex vivo and strategies where the manipulation takes place in vivo. In ex vivo protocols, cells are taken from the experimental animal or the patient (e.g., fibroblasts, hematopoietic cells, synoviocytes), are maintained and propagated in cell culture, transfected or transduced with foreign nucleic acid material, and finally, are given back to the same respective individual. This approach has some advantages: cell numbers can be increased by induction of proliferation, cells can be selected for successful gene transfer, and can be injected back at a specific location (e.g., into the synovium of a joint). The ex vivo technique is supposed to be reliable, however, it is highly labour intensive and thus is probably not the method of choice for future treatment of large numbers of patients. As opposed to the ex vivo strategies, in vivo application protocols directly transfer genetic material into the whole organism, in most cases with the help of a vector (modified virus) or liposomes. Therefore, cells of interest do not need to be isolated and grown in culture. Targeting the right cell types however, is a problem. Once this strategy is established, it should be easy and convenient in use. Still, the development of this approach is pretty much in its infancy (Evans et al., 1998; Gottschalk and Chan, 1998).

Since naked DNA is usually not efficiently taken up by cells, the choice of route of gene delivery is a major parameter which determines the outcome of gene transfer and subsequent gene expression. About 70% of all clinical trials in gene therapy have used viral vectors (Gottschalk and Chan, 1998). Of the non-viral delivery systems, cationic liposomes have been employed most often. Although use of liposomes gave encouraging results in vitro (Smith et al., 1993), their relevance for in vivo studies turned out to be limited due to their low stability in the blood compartment (Plank et al., 1996). Thus, gene therapy studies have focused on the use of viral vectors. Efficient transfection and high levels of gene expression can be reached using the viral vehicle. Particularly, modified forms of adenovirus, adeno-associated virus, and retroviruses derived from the Moloney murine leukemia virus have been used in clinical trials. The viruses are usually genetically disabled to reduce their pathogenicity. Problems of viral vectors still include antigenicity and insertional mutagenesis. Especially in the case of the adenovirus, inflammatory reactions have been reported (Bruder and Kovesdi, 1997; Clesham et al., 1998).

Of the inflammatory diseases in which the therapeutic potential of gene therapy has been investigated in animal models, rheumatoid arthritis is probably the best studied human disorder (Evans et al., 1998, 1999). It is obvious that these gene therapy approaches tie on with the results of studies using recombinant cytokines. Accordingly, blockage of biological activities of interleukin-1 and tumor necrosis factor α, as well as introduction of interleukin-10, interleukin-4, or interleukin-13 activity have become the focus of these studies.

Ex vivo retroviral mediated transfer of cDNA for interleukin-1 receptor antagonist to synoviocytes and subsequent local delivery back to the synovium resulted in prolonged (up to 6 weeks) intra-articular expression of interleukin-1 receptor antagonist protein (Evans et al., 1999) and turned out to be successful in animal models of rheumatoid arthritis. The studies revealed that this protocol caused a significant anti-arthritic effect with inhibition of matrix degradation, reduction of leukocyte infiltration, and decrease of joint swelling in a rabbit model of antigen-induced arthritis (Otani et al., 1996), in a rat model of bacterial cell wall-induced arthritis (Makarov et al., 1996), and in murine collagen- or zymosan-induced arthritis (Bakker et al., 1997). A different approach utilized local in vivo delivery of adenovirus in order to transfer the genes for soluble tumor necrosis factor receptor p55 and soluble interleukin-1 receptor I to the rabbit synovial lining. When transferred together, both genes mediated strong reduction of synovitis in the rabbit model of antigen-induced arthritis (Ghivizzani et al., 1998). Going one step further, systemic adenoviral delivery of soluble tumor necrosis factor receptor p55-immunoglobulin G was reported to mediate anti-arthritic effects in a rat model of collagen-induced arthritis (Le et al., 1997). A different approach was undertaken to investigate the effect of gene transfer for interleukin-4 and interleukin-13. Chinese hamster ovary cells were transfected with the genes coding for interleukin-4 or interleukin-13. Systemic inoculation significantly reduced the severity of collagen-induced arthritis in mice in both cases (Bessis et al., 1996). All these data from animal studies illustrate that in future gene therapy has the potential to become a powerful therapeutic alternative to conventional anti-inflammatory drugs. Phase I clinical trials are under way in which patient synovium is removed and ex vivo, retroviral gene transfer is performed with the cDNA coding for interleukin-1 receptor antagonist. Thereafter, cells are returned and expression of interleukin-1 receptor antagonist is assessed. Preliminary results show that expression of interleukin-1 receptor antagonist can be achieved successfully by gene transfer of complementary DNA into the human synovium (Ghivizzani et al., 1997). Future clinical trials will shed more light on efficacy, the clinical benefit and the safety of gene therapy for rheumatoid arthritis in humans.

7. Perspectives

The therapeutic potential of drugs that target transcription factors such as nuclear factor κB, nuclear factor of activated T-cells, activator protein-1 and others for treatment of acute and chronic inflammatory diseases or for immunosuppression seems very promising. The present

limitations may be overcome by detailed knowledge of the signalling cascades that uniquely serve to initiate and sustain pro- and anti-inflammatory gene expression. This may yield novel drugs that can prevent diseases such as rheumatoid arthritis rather than just relieving symptoms associated with these disorders.

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References

Abraham, E., Glauser, M.P., Butler, T., Garbino, J., Gelmont, D., Laterre, P.F., Knudsk, K., Bruining, H.A., Otto, C., Tobin, E., Zwingelstein, C., Lesslauer, W., Leighton, A., 1997. p55 tumor necrosis factor receptor fusion protein in the treatment of patients with severe sepsis and septic shock. A randomized controlled multicenter trial. Ro 45-2081 study group. JAMA 277, 1531–1538.

Arend, W.P., Malyak, M., Guthridge, C.J., Gabay, C., 1998. Interleukin-1 receptor antagonist: role in biology. Annu. Rev. Immunol. 16, 27–55.

Auphan, N., DiDonato, J.A., Rosette, C., Helmberg, A., Karin, M., 1995. Immunosuppression by glucocorticoids: inhibition of NF-κB activity through induction of IκB synthesis. Science 270, 286–290.

Baeuerle, P.A., 1998. Pro-inflammatory signaling: last pieces in the NF-κB puzzle?. Curr. Biol. 8, R19–R22.

Bakker, A., Joosten, L.A., Arntz, O.J., Helsen, M.M.A., Bendele, A.M., van de Loo, F.A.J., van den Berg, W.B., 1997. Prevention of murine collagen-induced arthritis in the knee and ipsilateral paw by local expression of human interleukin-1 receptor antagonist protein in the knee. Arthritis Rheum. 5, 893–900.

Ballow, M., Nelson, R., 1997. Immunopharmacology, immunomodulation and immunotherapy. JAMA 278, 2008–2017.

Barnes, P.J., Adcock, I., 1993. Anti-inflammatory actions of steroids: molecular mechanisms. Trends Pharmacol. Sci. 14, 436–441.

Bates, D.W., Sands, K., Miller, E., Lanken, P.N., Hibberd, P.L., Graman, P.S., Schwartz, J.S., Kahn, K., Snydman, D.R., Parsonnet, J., Moore, R., Black, E., Johnson, B.L., Jha, A., Platt, R., 1997. Predicting bacteremia in patients with sepsis syndrome. Academic medical center consortium sepsis project working group. J. Infect. Dis. 17, 1538–1551.

Berkman, N., John, M., Roesems, G., Jose, P.J., Barnes, P.J., Chung, K.F., 1995. Differential sensitivities in human blood monocytes and alveolar macrophages. J. Immunol. 155, 4412–4418.

Bessis, N., Boissier, M.-C., Ferrara, P., Blankenstein, T., Fradelizi, D., Fournier, C., 1996. Attenuation of collagen-induced arthritis in mice by treatment with vector cells engineered to secrete interleukin-13. Eur. J. Immunol. 26, 2399–2403.

Bogdan, C., Vodovotz, Y., Nathan, C., 1991. Macrophage deactivation by interleukin 10. J. Exp. Med. 174, 1549–1555.

Bresnihan, B., Alvaro-Gracia, J.M., Cobby, M., Doherty, M., Domljan, Z., Emery, P., Nuki, G., Pavelka, K., Rau, R., Rozman, B., Watt, I., Williams, B., Aitchison, R., McCabe, D., Musikic, P., 1998. Treatment of rheumatoid arthritis with recombinant human interleukin-1 receptor antagonist. Arthritis Rheum. 41, 2196–2204.

Briehl, M.M., Flomerfelt, F.A., Wu, X.P., Miesfeld, R.L., 1990. Transcriptional analyses of steroid-regulated gene networks. Mol. Endocrinol. 4, 287–294.

Brostjan, C., Anrather, J., Csizmadia, V., Stroka, D., Soares, M., Bach, F.H., Winkler, H., 1996. Glucocorticoid mediated repression of NF-κB activity in endothelial cells does not involve induction of IκB synthesis. J. Biol. Chem. 171, 19612–19616.

Bruder, J.T., Kovesdi, I., 1997. Adenovirus infection stimulates the Raf/MAPK signaling pathway and includes interleukin-8 expression. J. Virol. 71, 398–404.

Brun-Buisson, C., Doyon, F., Carlet, J., Dellamonica, P., Gouin, F., Lepoutre, A., Mercier, J.C., Offenstadt, G., Regnier, B., 1995. Incidence, risk factors, and outcome of severe sepsis and septic shock in adults. A multicenter prospective study in intensive care units. French ICU Group for Severe Sepsis. JAMA 274, 968–974.

Caelles, C., Ganzalez Sancho, J.M., Munoz, A., 1997. Nuclear hormone receptor antagonism with AP-1 by inhibition of the JNK pathway. Genes Dev. 11, 3351–3364.

Cain, B.S., Meldrum, D.R., Harken, A.H., McIntyre, R.C. Jr., 1998. The physiologic basis for anticytokine clinical trials in the treatment of sepsis. J. Am. Coll. Surg. 186, 337–350.

Caldenhoven, E., Liden, J., Wissink, S., Van de Stolpe, A., Raaijmakers, J., Koendermann, L. et al., 1995. Negative cross-talk between RelA and the glucocorticoid receptor: a possible mechanism for the anti-inflammatory action of glucocorticoids. Mol. Endocrinol. 9, 401–412.

Cato, A.C.B., Wade, E., 1996. Molecular mechanisms of anti-inflammatory action of glucocorticoids. BioEssays 18, 371–378.

Clesham, G.J., Adam, P.J., Proudfoot, D., Flynn, P.D., Efstathiou, S., Weissberg, P.L., 1998. High adenoviral loads stimulate NFκB-dependent gene expression in human vascular smooth muscle cells. Gene Ther. 5, 174–189.

Cope, A.P., 1998. Regulation of autoimmunity by proinflammatory cytokines. Curr. Opin. Immunol. 10, 669–676.

Dalman, F.C., Scherrer, L.C., Taylor, L.P., Akil, H., Pratt, W.B., 1991. Localisation of the 90-kDa heat shock protein-binding site within the hormone-binding domain of the glucocorticoid receptor by peptide competition. J. Biol. Chem. 266, 3482–3490.

DeWitt, D.L., Meade, E.A., 1993. Serum and glucocorticoid regulation of gene transcription and expression of the prostaglandin H synthase-2 isozymes. Arch. Biochem. Biophys. 306, 94–102.

Dinarello, C.A., 1996. Biologic basis for interleukin-1 in disease. Blood 87, 2095–2147.

Dinarello, C.A., Gelfand, J.A., Wolff, S.M., 1993. Anticytokine strategies in the treatment of the systemic inflammatory response syndrome. JAMA 269, 1829–1835.

Dinarello, C.A., Novick, D., Puren, A.J., Fantuzzi, G., Shapiro, L., Mühl, H., Yoon, D.-Y., Reznikov, L.L., Kim, S.-H., Rubinstein, M., 1998. Overview of interleukin-18: more than an interferon-γ inducing factor. J. Leukocyte Biol. 63, 658–664.

Doherty, G.M., Lange, J.R., Langstein, H.N., Alexander, H.R., Buresh, C.M., Norton, J.A., 1992. Evidence for IFN-γ as a mediator of the lethality of endotoxin and tumor necrosis factor-α. J. Immunol. 149, 1666–1670.

Eigler, A., Sinha, B., Hartmann, G., Endres, S., 1997. Taming TNF: strategies to restrain this proinflammatory cytokine. Immunol. Today 10, 487–492.

Evans, C.H., Ghivizzani, S.C., Robbins, P.D., 1998. Blocking cytokines with genes. J. Leukocyte Biol. 64, 55–61.

Evans, C.H., Ghivizzani, S.C., Kang, R., Muzzonigro, T., Wasko, M.C., Herndon, J.H., Robbins, P.D., 1999. Gene therapy for rheumatic diseases. Arthritis Rheum. 42, 1–16.

Firestein, G.S., Zvaifler, N.J., 1997. Anticytokine therapy in rheumatoid arthritis. N. Engl. J. Med. 337, 195–197.

Fisher, C.J. Jr., Agosti, J.M., Opal, S.M., Lowry, S.F., Balk, R.A., Sadoff, J.C., Abraham, E., Schein, R.M.H., Benjamin, E., 1996. N. Engl. J. Med. 334, 1697–1702.

Frantz, B., Nordby, Bren, G., Steffan, N., Paya, C.V., Kincaid, R.L., Tocci, M.J., O'Keefe, S.J.O., O'Neill, E.A.O., 1994. Calcineurin acts in synergy with PMA to inactivate IκB/MAD3, an inhibitor of NF-κB. EMBO J. 13, 861–870.

Fuchs, A.C., Granovitz, E.V., Shapiro, L., Vannier, E., Lonnemann, G.,

Angel, J.B., Kennedy, J.S., Rabson, A.R., Radwanski, E., Affrime, M.B., Cutler, D.L., Grint, P.C., Dinarello, C.A., 1996. Clinical, hematologic, and immunologic effects of interleukin-10 in humans. J. Clin. Immunol. 16, 291–303.

Ghivizzani, S.C., Kang, R., Muzzonigro, T., Whalen, J., Watkins, S.C., Herndon, J.H. et al., 1997. Gene therapy for arthritis-treatment of the first three patients. Arthritis Rheum. 40, 223.

Ghivizzani, S.C., Lechman, E.R., Kang, R., Tio, C., Kollis, J., Evans, C.H., Robbins, P.D., 1998. Direct adenovirus-mediated gene transfer of interleukin 1 and tumor necrosis factor α soluble receptors to rabbit knees with experimental arthritis has local and distal anti-arthritic effects. Proc. Natl. Acad. Sci. U.S.A. 95, 4613–4618.

Gottesfeld, J.M., Neely, L., Trauger, J.W., Baird, E.E., Devan, P.B., 1997. Regulation of gene expression by small molecules. Nature 387, 202–205.

Gottschalk, U., Chan, S., 1998. Somatic gene therapy. Present situation and future perspective. Arzneim.-Forsch./Drug Res. 48, 1111–1120.

Granowitz, E.V., Porat, R., Mier, J.W., Pribble, J.P., Stiles, D.M., Bloedow, D.C., Catalano, M.A., Wolff, S.M., Dinarello, C.A., 1992. Pharmacokinetics, safety and immunomodulatory effects of human recombinant interleukin-1 receptor antagonist in healthy humans. Cytokine 4, 353–360.

Guido, E.C., Delorme, E.O., Clemm, D.L., Stein, R.B., Rosen, J., Miner, J.N., 1996. Determinants of promoter-specific activity by glucocorticoid receptor. Mol. Endocrinol. 10, 1178–1190.

Heck, S., 1994. A distinct modulating domain in glucocorticoid receptor monomers in the repression of activity of the transcription factor AP-1. EMBO J. 13, 4087–4095.

Hojo, M., Morimoto, T., Maluccio, M., Asano, T., Morimoto, K., Lagman, M., Shimbo, T., Suthanthiran, M., 1999. Cyclosporine induces cancer progression by a cell-autonomous mechanism. Nature 397, 530–534.

Isomaki, P., Punnonen, J., 1997. Pro- and anti-inflammatory cytokines in rheumatoid arthritis. Ann. Med. 29, 499–507.

Jonat, C., Rahmsdorf, H.J., Park, K.K., Cato, A.C., Gebel, S., Ponta, H., Herrlich, P., 1990. Antitumor promotion and anti-inflammation: down-modulation of AP-1 (Fos/Jun) activity by glucocorticoid hormone. Cell 62, 1189–1204.

Kamei, Y., Xu, L., Heinzel, T., Torchia, J., Kurokawa, R., Gloss, B., Lin, S.C., Heyman, R.A., Rose, D.W., Glass, C.K., Rosenfeld, M.G., 1996. A CBP integrator complex mediates transcriptional activation and AP-1 inhibition by nuclear receptors. Cell 85, 403–414.

Knudsen, P.J., Dinarello, C.A., Strom, T.B., 1986. Prostaglandins post-transcriptionally inhibit monocyte expression of interleukin 1 activity by increasing intracellular cyclicadenosine monophosphate. J. Immunol. 137, 3189–3194.

Kopp, E., Sankar, G., 1994. Inhibition of NF-κB binding by sodium salicylate and aspirin. Science 265, 956–959.

Kunz, D., Walker, G., Eberhardt, W., Nitsch, D., Pfeilschifter, J., 1995. Interleukin-1β-induced expression of nitric oxide synthase in rat renal mesangial cells is suppressed by cyclosporin A. Biochem. Biophys. Res. Commun. 216, 438–446.

Kunz, D., Walker, G., Eberhardt, W., Pfeilschifter, J., 1996. Molecular mechanisms of dexamethasone inhibition of nitric oxide synthase expression in interleukin 1β-stimulated mesangial cells: evidence for the involvement of transcriptional and posttranscriptional regulation. Proc. Natl. Acad. Sci. U.S.A. 93, 255–259.

Lalani, I., Bhol, K., Ahmed, A.R., 1997. Interleukin-10: biology, role in inflammation and autoimmunity. Ann. Allergy, Asthma, Immunol. 79, 469–483.

Le, C.H., Nicolson, A.G., Morales, A., Sewell, K.L., 1997. Suppression of collagen-induced arthritis through adenovirus-mediated transfer of a modified tumor necrosis factor α receptor gene. Arthritis Rheum. 40, 1662–1669.

Liden, J., Delaunay, F., Rafter, I., Gustafsson, J., Okret, S., 1997. A new function for the C-terminal zinc finger of the glucocorticoid receptor. Repression of RelA transactivation. J. Biol. Chem. 272, 21467–21472.

Liu, J., 1993. FK506 and cyclosporin, molecular probes for studying intracellular signal transduction. Immunol. Today 14, 290–295.

Makarov, S.S., Olsen, J.C., Johnston, W.N., Anderle, S.K., Brown, R.R., Baldwin, A.S. Jr., Haskill, J.S., Schwab, J.H., 1996. Suppression of experimental arthritis by gene transfer of interleukin 1 receptor antagonist cDNA. Proc. Natl. Acad. Sci. U.S.A. 93, 402–406.

Marienfeld, R., Neumann, M., Chuvpilo, S., Escher, C., Kneitz, B., Avots, A., 1997. Cyclosporin A interferes with the inducible degradation of NF-κB inhibitors, but not with the processing of p105/NF-κB1 in T-cells. Eur. J. Immunol. 27, 1601–1609.

May, M.J., Gosh, S., 1998. Signal transduction through NF-κB. Immunol. Today 19, 80–88.

McEwan, I.J., Wright, A.P.H., Gustafsson, J.-A., 1997. Mechanism of gene expression by the glucocorticoid receptor: role of protein–protein interactions. BioEssays 19, 153–160.

Meyer, S., Kohler, N.G., Joly, A., 1997. Cyclosporin A is an uncompetitive inhibitor of proteosome activity and prevents NF-κB activation. FEBS Lett. 413, 354–358.

Moreland, L.W., Baumgartner, S.W., Schiff, M.H., Tindall, E.A., Fleischmann, R.M., Weaver, A.L., Ettlinger, R.E., Cohen, S., Koopman, W.J., Mohler, K., Widmer, M.B., Blosch, C.M., 1997. Treatment of rheumatoid arthritis with a recombinant human tumor necrosis factor receptor (p75)-Fc fusion protein. N. Engl. J. Med. 337, 141–147.

Mühl, H., Geiger, T., Pignat, W., Märki, F., van den Bosch, H., Cerletti, N., Cox, D., McMaster, G., Vosbeck, K., Pfeilschifter, J., 1992. Transforming growth factors type-β and dexamethasone attenuate group II phospholipase A_2 gene expression by interleukin 1 and forskolin in rat mesangial cells. FEBS Lett. 301, 190–194.

Mühl, H., Kunz, D., Rob, P., Pfeilschifter, J., 1993. Cyclosporin derivatives inhibit interleukin 1β induction of nitric oxide synthase in renal mesangial cells. Eur. J. Pharmacol. 249, 95–100.

Novick, D., Kim, S.-H., Fantuzzi, G., Reznikov, L.L., Dinarello, C.A., Rubinstein, M., 1999. Interleukin-18 binding protein: a novel modulator of the Th1 cytokine response. Immmunity 10, 127–136.

Okamura, H., Tsutsui, H., Komatsu, T., Yutsudo, M., Hakura, A., Tanimoto, T., Torigoe, K., Okura, T., Nukada, Y., Hattori, K., Akita, K., Namba, M., Tanabe, F., Konishi, K., Fukuda, S., Kurimoto, M., 1995. Cloning of a new cytokine that induces IFN-γ production by T cells. Nature 378, 88–91.

Opal, S.M., Fisher, C.J., Dhainaut, J.-F., Vincent, J.-L., Brase, R., Lowry, S.F., Sadoff, J.C., Slotman, G.J., Levy, H., Balk, R.A., Shelly, M.P., Pribble, J.P., LaBrecque, J.F., Lookabaugh, J., Donovan, H., Dubin, H., Baughman, R., Norman, J., Demaria, E., Matzel, K., Abraham, E., Seneff, M., 1997. Confirmatory interleukin-1 receptor antagonist trial in severe sepsis: a phase III, randomized, double-blind, placebo-controlled, multicenter trial. Crit. Care Med. 25, 1115–1124.

Otani, K., Nita, I., Macaulay, W., Gerorgescu, H.I., Robbins, P.D., Evans, C.H., 1996. Suppression of antigen-induced arthritis in rabbits by ex vivo gene therapy. J. Immunol. 156, 3558–3562.

Owens, G.P., Hahn, W.E., Cohen, J.J., 1991. Identification of mRNAs associated with programmed cell death in immature thymocytes. Mol. Cell Biol. 11, 4177–4188.

Papavassiliou, A.G., 1998. Transcription-factor-modulating agents: precision and selectivity in drug design. Mol. Med. Today 4, 358–366.

Peterson, M.G., Baichwal, V.R., 1993. Transcription factor based therapeutics: drugs of the future?. TIBTECH 11, 11–18.

Pfahl, M., 1993. Nuclear receptor/AP-1 interaction. Endocrinol. Rev. 14, 651–658.

Pfeilschifter, J., Schwarzenbach, H., 1990. Interleukin 1 and tumor necrosis factor stimulate cGMP in rat renal mesangial cells. FEBS Lett. 273, 185–187.

Pfeilschifter, J., Vosbeck, K., 1991. Transforming growth factor β_2 inhibits interleukin 1β-and tumour necrosis factor α-induction of nitric oxide synthase in rat renal mesangial cells. Biochem. Biophys. Res. Commun. 175, 372–379.

Pfeilschifter, J., Pignat, W., Leighton, J., Märki, F., Vosbeck, K., Alkan, S., 1990. Transforming growth factor β_2 differentially modulates

interleukin 1β- and tumour necrosis factor α-stimulated phospholipase A_2 and prostaglandin E_2 synthesis in rat renal mesangial cells. Biochem. J. 270, 269–271.

Plank, C., Mechtler, K., Szoka, F.C., Wagner, E., 1996. Activation of the complement system by synthetic DNA complexes: a potential barrier for intravenous gene delivery. Hum. Gene Ther. 7, 1437–1446.

Puren, A.J., Fantuzzi, G., Gu, Y., Su, M.S.-S., Dinarello, C.A., 1998. Interleukin-18 (IFNγ-inducing factor) induces IL-8 and IL-1β via TNFα production from non-$CD14^+$ human blood mononuclear cells. J. Clin. Invest. 101, 711–721.

Ray, A., Prefontaine, K.E., 1994. Physical association and functional antagonism between the p65 subunit of transcription of NF-κB and the glucocorticoid receptor. Proc. Natl. Acad. Sci. U.S.A. 91, 752–756.

Reichardt, H.M., Schütz, G., 1998. Glucocorticoid signalling — multiple variations of a common theme. Mol. Cell. Endocrinol. 146, 1–6.

Schalkwijk, C., Vervoordeldonk, M., Pfeilschifter, J., Märki, F., van den Bosch, H., 1991. Cytokine- and forskolin-induced synthesis of group II phospholipase A_2 and prostaglandin E_2 in rat mesangial cells is prevented by dexamethasone. Biochem. Biophys. Res. Commun. 180, 46–52.

Schalkwijk, C., Pfeilschifter, J., Märki, F., van den Bosch, H., 1992. Interleukin 1β- and Forskolin-induced synthesis and secretion of group II phospholipase A_2 and prostaglandin E_2 in rat mesangial cells is prevented by transforming growth factor β2. J. Biol. Chem. 267, 8846–8851.

Schalkwijk, C., Vervoordeldonk, M., Pfeilschifter, J., van den Bosch, H., 1993. Interleukin 1 β-induced cytosolic phospholipase A_2 activity and protein synthesis is blocked by dexamethasone in rat mesangial cells. FEBS Lett. 333, 339–343.

Scheinman, R.I., Cogswell, P.C., Lofquist, A.K., Baldwin, A.S. Jr., 1995. Role of transcriptional activation of IκBα in mediation of immunosuppression by glucocorticoids. Science 270, 283–286.

Schreiber, S.L., Crabtree, G.R., 1992. The mechanism of action of cyclosporin A and FK506. Immunology Today 13, 136–142.

Schüle, R., Rangarajan, P., Kliewer, S., Ransone, L.J., Bolado, J., Yang, N., Verma, I.M., Evans, R.M., 1990. Functional antagonism between oncoprotein c-Jun and the glucocorticoid receptor. Cell 62, 1217–1226.

Schwenger, P., Bellosta, P., Vietor, I., Basilico, C., Skolnik, E.Y., Vilcek, J., 1997. Sodium salicylate induces apoptosis via p38 mitogen-activated protein kinase but inhibits tumor necrosis factor-induced c-Jun N-terminal kinase/stress-activated protein kinase activation. Proc. Natl. Acad. Sci. U.S.A. 94, 2869–2873.

Smith, J.G., Walzem, R.L., German, J.B., 1993. Liposomes as agents of DNA transfer. Biochim. Biophys. Acta 21, 327–340.

Stein, C.A., 1996. Exploiting the potential of antisense: beyond phosphorothioate oligodeoxynucleotides. Chem. Biol. 3, 319–323.

Tetta, C., Mariano, F., Buades, J., Ronco, C., Wratten, M.L., Camussi, G., 1997. Relevance of platelet-activating factor in inflammation and sepsis: mechanisms and kinetics of removal in extracorporeal treatments. Am. J. Kidney Dis. 30, S57–S65.

van der Meer, J.W., Vogels, M.T., Netea, M.G., Kullberg, B.J., 1998. Proinflammatory cytokines and treatment of disease. Ann. N.Y. Acad. Sci. 856, 243–251.

Vannier, E., Miller, L.C., Dinarello, C.A., 1992. Coordinated anti-inflammatory effects of interleukin 4: interleukin 4 suppresses interleukin 1 production but up-regulates gene expression and synthesis of interleukin 1 receptor antagonist. Proc. Natl. Acad. Sci. U.S.A. 89, 4076–4080.

Vannier, E., de Waal Malefyt, R., Salazar-Montes, A., de Vries, J.E., Dinarello, C.A., 1996. Interleukin-13 (IL-13) induces IL-1 receptor antagonist gene expression and protein synthesis in peripheral blood mononuclear cells: inhibition by an IL-4 mutant protein. Blood 87, 3307–3315.

van Roon, J.A.G., van Roy, J.L.A.M., Gmelig-Meyling, F.H.J., Lafeber, F.P.J.G., Bijlsma, J.W.J., 1996. Prevention and reversal of cartilage degradation in rheumatoid arthritis by interleukin-10 and interleukin-4. Arthritis Rheum. 39, 829–835.

Walker, G., Pfeilschifter, J., Kunz, D., 1997a. Mechanisms of suppression of inducible nitric oxide synthase (iNOS) expression in interferon (IFN)-γ-stimulated RAW 264.7 cells by dexamethasone. Evidence for glucocorticoid-induced degradation of iNOS protein by calpain as a key step in post-transcriptional regulation. J. Biol. Chem. 272, 16679–16687.

Walker, G., Kunz, D., Pignat, W., van den Bosch, H., Pfeilschifter, J., 1997b. Suppression by cyclosporin A of interleukin 1β-induced expression of group II phospholipase A_2 in rat renal mesangial cells. Br. J. Pharmacol. 121, 787–793.

Wilckens, T., de Rijk, R., 1997. Glucocorticoids and immune function: unknown dimensions and new frontiers. Immunol. Today 18, 418–424.

Wissink, S., van Heerde, E.C., Schmitz, M.L., Kalkhoven, E., van der Burg, B., Baeuerle, P.A., van der Saag, P.T., 1997. Distinct domains of the RelA NF-κB subunit are required for negative cross-talk and direct interaction with the glucocorticoid receptor. J. Biol. Chem. 272, 22278–22284.

Wolkow, P.P., 1998. Involvement and dual effects of nitric oxide in septic shock. Inflammation Res. 47, 152–166.

Yang Yen, H.F., Chambard, J.C., Sun, Y.L., Smeal, T., Schmidt, T.J., Drouin, J., Karin, M., 1990. Transcriptional interference between c-Jun and the glucocorticoid receptor: mutual inhibition of DNA binding due to direct protein–protein interaction. Cell 62, 1205–1215.

Yin, M.-J., Yamamato, Y., Gaynor, R.B., 1998. The anti-inflammatory agents aspirin and salicylate inhibit the activity of IκB kinase-β. Nature 396, 77–80.

Zilliacus, J., Wright, A.P., Carlstedt-Duke, J., Gustafsson, J.-A., 1995. Structural determinants of DNA-binding specificity by steroid receptors. Mol. Endocrinol. 9, 389–400.