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REVIEW

Inducible nitric oxide synthase in human diseases

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INTRODUCTION

Since its discovery as a biologically active molecule in the late 1980s, nitric oxide (NO) has been found to play an important role as signal molecule in many parts of the organism as well as cytotoxic or regulatory effector molecule of the innate immune response. The signal molecule NO is synthesized on demand for short periods of time (seconds to minutes) following enzyme activation of constitutively expressed endothelial NO synthase (eNOS) or neuronal NO synthase (nNOS). In contrast, the inducible NO synthase (iNOS) is expressed after cell activation only and then produces NO for comparatively long periods of time (hours to days). Thus, regulated short pulsative synthesis *versus* constant NO production differentiates between physiological and pathophysiological actions of NO (for review see [1]). As human monocytes in contrast to rodent ones do not produce large amounts of NO when activated *in vitro*, iNOS expression in human diseases has long been questionable. However, in the last 3 years data have accumulated on iNOS expression in a variety of human diseases or disorders. We here try to review our current understanding of the role of iNOS in human diseases.

MOLECULAR BIOLOGY OF HUMAN iNOS

The iNOS gene is under the transcriptional control of a variety of inflammatory mediators such as cytokines, lipopolysaccharide (LPS), and others (for review see [2]). iNOS cDNAs have independently been cloned from several tissues with only small differences in the deduced amino acid sequences [3–6]. The overall nucleotide sequence identity between human and murine iNOS cDNA is about 80% [3]. Molecular cloning revealed that the iNOS gene is about 37 kb in length [7] and is located on chromosome 17 at position 17cen-q11.2 [8]. The iNOS open reading frame is encoded by 27 exons, with translation initiation and termination in exons 2 and 27, respectively [9]. All intron/exon boundaries of the human iNOS gene conform strictly to the known GT/AG donor/acceptor rule. The structure of the coding region, especially of the cofactor binding sites, is very similar to those of human nNOS [10] and eNOS [11,12]. Southern blot analysis revealed single bands for nNOS and eNOS but multiple bands for iNOS exons 22–26 in humans and apes [13,14] when using the 3′ end of an iNOS cDNA probe. An unprocessed, highly mutated pseudogene has been localized on the same chromosomal region as the functional iNOS gene by fluorescence *in situ* hybridization (FISH) analysis [15]. Three minor allelic variants have been described [16–18], one bearing pathophysiological significance in resistance against malaria infection [18].

The high homology of iNOS isoforms among different species and various cell types suggests that they are all products of the same gene. However, human iNOS gene transcription in distinct cells is reported to be regulated differently. Significant differences between the human and the murine iNOS promoter region were found by 3′ analysis. Only 1·5 kb of the proximal 5′ flanking region of the murine promoter are necessary to confer inducibility to LPS and interferon-gamma (IFN-γ), whereas the human iNOS promoter is hyporesponsive to LPS/IFN-γ due to nucleotide exchanges in the LPS/IFN-γ-responsive enhancer region (-1083 to -1229) [19,20]. Additionally, the human transcription factor NF-κB, induced by treatment with IL-1β, tumour necrosis factor-alpha (TNF-α) and IFN-γ, binds to the iNOS promoter more weakly than mouse NF-κB does [21]. However, three regions with cytokine-responsive cis-regulatory elements (lying between $-3{\cdot}8$ kb and -16 kb in the promoter region) confer cytokine inducibility [22,23]. Interestingly, the human iNOS gene contains a shear-stress responsive element (GAGACC) which is identical to that in human eNOS, but this element does not exist in the murine iNOS promoter [24]. Induction of NO production by this shear-stress element seems to be a key mediator for protection of cardiovascular diseases via inhibition of leucocyte adhesion, platelet aggregation, and vascular smooth muscle cell (VSMC) proliferation. At position -226 to -212 an element containing a sequence homology to the human hypoxia-responsive element (HRE) [25] was found in the murine promoter conferring iNOS induction at decreased oxygen tension in IFN-γ-activated macrophages [26]. Induction of the hypoxia-inducible factor-1 by hypoxia and binding to the iNOS-HRE in cooperation with IFN-γ leads to iNOS induction. The necessity for IFN-γ costimulation may help to limit iNOS expression to inflammatory sites with hypoxic conditions. Although the activity of the human HRE is questionable due to two mismatches in the consensus sequence, culture of human hepatoma cells under hypoxic conditions indeed led to iNOS induction [27]. In conclusion, the human iNOS promoter is one of the largest and most complex promoters known today (Fig. 1a,b), indicative of a tightly controlled iNOS gene expression.

A TATA box is located 30 bp upstream of the transcription start site and exon 2 contains the ATG initiation codon which lies in a Kozak consensus sequence, but about 6% of the cytokine-inducible iNOS transcripts in human macrophages and epithelial cells start at multiple transcription initiation sites (Fig. 1a), some extending several hundred base pairs upstream from the main TATA-directed initiation site [28]. Further diversity in the iNOS mRNAs is gained

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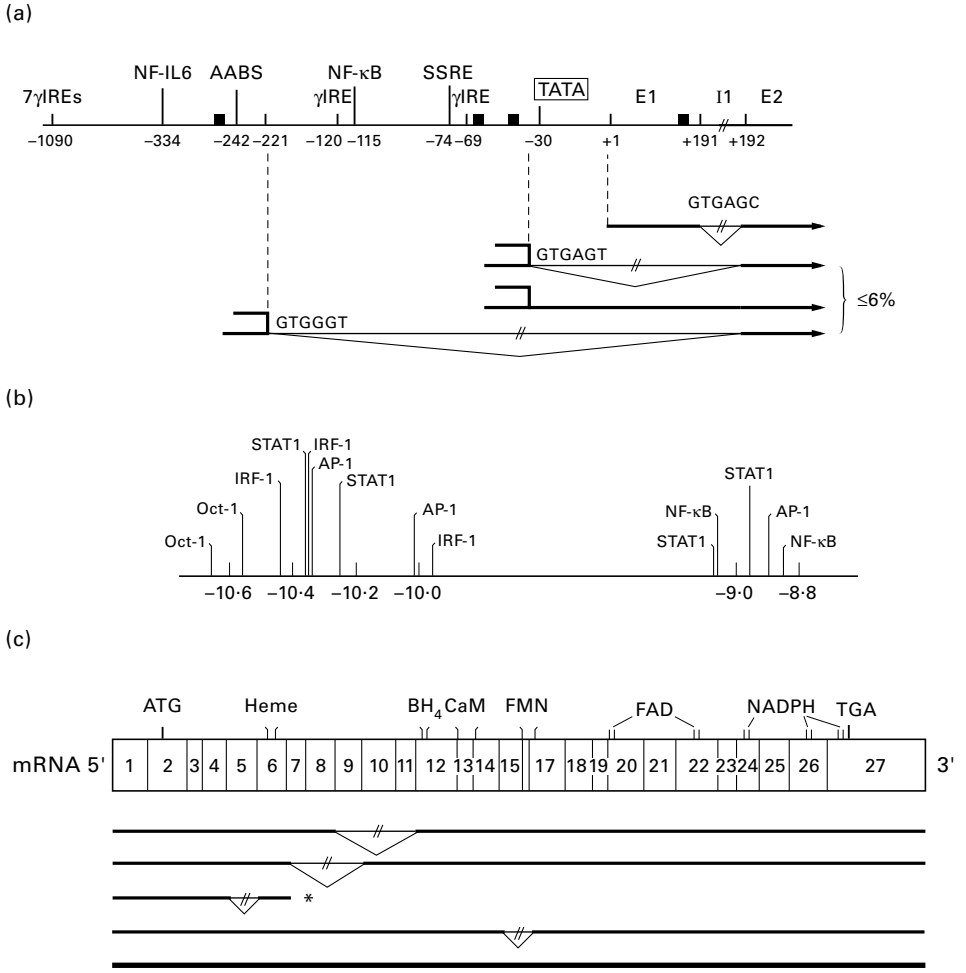

Fig. 1. Schematic structure of the human inducible nitric oxide synthase (iNOS) 5′ flanking region (a), the upstream enhancer region (b), and the human iNOS mRNA (c). (a) The main transcriptional start site is denoted at position + 1. Several potential transcription factor binding sites are indicated. The TATA box begins at −30. TATA-independent iNOS transcripts have alternative splice sites at positions −221, −36 and +191 in the 5′ UTR of the gene. Possible start codons (■) of open reading frames are located at −256, −65, −45, −40 and +187. (b) Structure of the distal part of the human iNOS promoter which seems to be a cytokine-responsive enhancer element. This promoter region (−10·9 to −8·7 kb) increases iNOS transcription orientation independently by a factor of 2. It contains multiple binding sites for transcription factors, which are activated in response to either IFN-γ (IRF-1, STAT1) or IL-1β (AP-1, IRF-1). (c) Alternative splicing of human iNOS mRNA. The ratios of alternatively to constitutively spliced mRNA differ among tissues and depend on activation by cytokines. *Truncated iNOS with exon 5 deletion abundant in human cerebellum [29]. γIRE, IFN-γ-responsive element; NF, nuclear factor; AABS, activator binding site; SSRE, shear stress responsive element; E, exon; I, intron; IRF, interferon regulatory factor; STAT, signal transducer and activator transcription.

by alternative splicing. Five distinct alternative splicing regions have been found (Fig. 1c), one of which leads to the distinct deletion of exon 5 with a translational frame shift leading to a stop codon in exon 6 yielding a premature iNOS product of 134 amino acids. This deletion is abundant in cerebellum, suggesting a specific tissue-related function. Losses of exon 8 and 9, exon 9–11, or exon 15 and 16 by alternative splicing are in frame deletions [29]. Exon 15–16 deletion leads to iNOS proteins missing the FMN binding site. Alternative splicing of exon 1 together with the different transcription initiation sites leads to variable lengths of the 5′ untranslated region (UTR) in a minor fraction of the iNOS mRNA [28]. Human iNOS mRNA has a long and complex 5′UTR containing eight partially overlapping open reading frames prior to the start codon AUG. For other genes open reading frames in the 5′UTR of a specific RNA have been shown to inhibit its translation in a tissue-specific manner [30]. Moreover, the 3′UTRs in exon 27 of both human and murine iNOS mRNA also bear regulatory functions [31]. Both contain a UUAUUUAU motif that is common to a variety of cytokine and oncogene mRNAs [32]. This motif has been shown to confer RNA instability, resulting in rapid degradation [33] (thereby lowering basal promoter activity in transfection studies [34]). Comparison of the 3′ ends of the iNOS cDNAs from murine and human cells revealed poor sequence conservation within the 3′UTR except for these AU segments. The 3′UTR of murine iNOS mRNA contains two of these copies, while the human mRNA contains two additional elements. At least six nucleotides of these copies match the consensus motif. Rapid degradation due to the conserved AU-rich octanucleotide sequences results in transient expression of iNOS mRNA with a half life of about 6 h in murine cells. In the RAW 264 macrophage

cell line two different 3′ ends have been found [35], indicating that mRNAs with different stabilities may be produced via alternative splicing. Exclusively in the human iNOS gene, the poly(A) signal, a GT-rich region, is located 10 bp downstream from the poly(A) site in the 3′ flanking region, while the usual poly(A) signal (AATAAA) is missing [34]. Differences in gene expression and mRNA stability due to these two distinct signals are still not yet known.

Obviously, further extensive studies are necessary to characterize all regulatory elements and transcription factors involved in transcriptional and post-transcriptional regulation of human iNOS gene expression. Current data indicate that its regulation reflects considerable complexity and tissue specificity.

iNOS IN HUMAN INFECTIOUS DISEASES

In rodents, NO produced by activated macrophages via iNOS has been found to play a major role as antiparasitic cytotoxic effector molecule (for review see [36]). Although it is now established that human macrophages are able to express iNOS, the relevance of macrophage-produced NO in human infectious diseases still has to be elucidated (for reviews see [37–39]). Data concerning iNOS expression in human viral or bacterial infections are summarized in Table 1. In addition, *in vitro* killing via NO of *Mycobacterium avium-intracellulare*, *Trypanosoma cruzi* and *Leishmania major* by activated human macrophages has been found [49–51], as well as growth inhibition of *Cryptococcus neoformans* by activated human astrocytes [52]. Cytokine-activated human neutrophils contain the iNOS protein and mediate tyrosine nitration of ingested *Staphylococcus aureus* and *Escherichia coli* [53]. This proves iNOS expression during a variety of infectious diseases, but whether iNOS activity plays a dominant role in the combatting of pathogens in humans is still under debate.

There are good indications for a key defence role for NO at the interface between the human and the external environment. On the surface of the tongue facultative anaerobic bacteria reduce nitrate of the saliva rapidly to nitrite, which when swallowed will generate NO in the micromolar range due to the acidic conditions of the stomach [54]. Also, NO is continuously released from human skin surfaces. Patients on long-term antibiotic therapy show reduced NO generation, thus skin commensal bacteria are thought to reduce sweat nitrate to nitrite, which is subsequently reduced non-enzymatically to NO again due to the acidic conditions on the skin surface [55]. Interestingly, in nasal airways of healthy subjects iNOS has been found to be constitutively expressed (or continuously induced) apically in the epithelial cells of paranasal sinuses [56]. Sinus air contains NO in concentrations close to the highest permissible atmospheric pollution levels [57]. All these findings suggest that NO indeed plays a role in the human defence against invading pathogens.

Studies with animals have shown that increased NO production contributes to excessive vasodilation during endotoxic and cytokine-induced shock. In patients with septic shock, plasma NO_x and nitrotyrosine concentrations are increased, and application of low doses of a specific NOS inhibitor partially reverses the widespread decrease in vascular tone as well as the fall in blood pressure, but it also produced a decrease in cardiac output (for reviews see [58,59]). However, additional data regarding iNOS expression in various organs, NO production by constitutive NOS *versus* iNOS, and differences in the role of NO in early *versus* late stages of shock are still necessary for understanding the role of iNOS activity in this disease.

iNOS IN HUMAN AUTOIMMUNE AND CHRONICALLY INFLAMMATORY DISEASES

Using immunocytochemistry, reverse transcriptase-polymerase chain reaction (RT-PCR), and *in situ* hybridization, iNOS expression has been described in rheumatoid arthritis (RA), multiple sclerosis (MS), and Sjögren's syndrome (Table 2). The NO oxidation product nitrite was found to be six- to 35-fold increased in the synovial fluid of RA patients compared with patients with osteoarthritis [89,90]. In active demyelinating lesions of MS patients, macrophages were found to stain for iNOS protein [63,64] and nitrotyrosine [91], indicative of nitrosative stress. These cells were found to produce high-output NO when isolated and cultured *in vitro* [64]. Type-1 diabetes, the most prevalent human immune-mediated disease, is the result of >90% destruction of the pancreatic islet mass. Data concerning early human prediabetic stages do not exist, but excellent animal models (BB rats and NOD mice) are available which spontaneously develop diabetes closely resembling the human disease. In these animal models, iNOS protein has been detected in macrophage islet infiltrates during early disease stages [92,93]. Rat islet cells are extremely prone to NO-induced cell death [94–96]. At least part of the islet-specific toxicity of streptozotocin is due to intracellular release of its NO moiety [97], and streptozotocin is known to be extremely diabetogenic in humans as well. Isolated human islets are also lysed by NO, albeit at higher concentrations [98], and the human cells can also be activated to express iNOS mRNA and to

Table 1. Inducible nitric oxide synthase (iNOS) expression in human infectious diseases

Infectious agent/infection	Localization of iNOS	References
HIV	Brain cortex	[40]
	Cytomegalovirus-infected retina	[41]
	Macrophages in dorsal root ganglia	[42]
Helicobacter pylori	Macrophages, EC of gastric wall	[43]
	Polymorphonuclear leucocytes, mononuclear cells	[44]
Mycobacterium tuberculosis	Alveolar macrophages	[45]
Respiratory tract infection	Inflammatory cells in nasal mucosa	[46]
Urinary tract infection	Neutrophils in urine	[47]
Malaria	Peripheral blood mononuclear cells	[48]

EC, Endothelial cells.

Table 2. Inducible nitric oxide synthase (iNOS) expression and cytokine profile in human autoimmune and chronically inflammatory diseases

Disease	Cytokine profile	Localization of iNOS	Reference
Rheumatoid arthritis	IL-1, IL-6, IL-8, TNF-α, GM-CSF	Synovial lining cells, EC, mononuclear cells, fibroblasts, VSMC	[60–62]
Multiple sclerosis	IL-1, IL-2, IL-6, TNF-α, IFN-γ	Macrophages, microglia	[63,64]
Sjögren's syndrome	IL-1β, IL-6, TNF-α, IFN-γ	Acinar + ductal epithelial cells	[65]
Asthma	IL-1β, IL-8, TNF-α, GM-CSF	Epithelium, some inflammatory cells	[66]
Bronchiectasis	IL-1β, IL-8, TNF-α	Macrophages	[67]
Idiopathic pulmonary fibrosis	IL-1β, IL-6, IL-8, TNF-α, IFN-γ, TGF-β	Macrophages, neutrophils, airway + alveolar epithelium	[68]
Atherosclerotic plaques	IL-1, IL-6, IL-12, TNF-α, IFN-γ	Macrophages, foam cells, VSMC	[69,70]
Ulcerative colitis	IL-1, TNF-α, IFN-γ	Epithelial cells, inflammatory infiltrate	[71–74]
		Epithelial cells	[75]
Crohn's disease	IL-1, TNF-α, IFN-γ	Epithelial cells, inflammatory infiltrate	[73]
		Epithelial cells	[75]
Necrotizing enterocolitis	IL-6, TNF-α, IFN-γ	Epithelial cells	[76]
Coeliac disease	IL-2, IL-6, TNF-α, IFN-γ	Epithelial cells, macrophages	[77]
Glomerulonephritis	TNF-α, IFN-γ	Macrophages	[78]
Dilated cardiomyopathy	IL-6, IL-8, TNF-α	Myocytes	[79–81]
		Myocytes, EC, VSMC	[82]
Psoriasis	IL-1β, IL-8	Keratinocytes	[83]
Cutaneous lupus erythematosus	IL-1β, IL-6, TNF-α	Basal epidermal layer	[84]
Systemic lupus erythematosus	IL-6, TNF-α	EC, keratinocytes	[85]
Systemic sclerosis	IL-1β, IL-6, IL-8, TNF-α	EC, fibroblasts, macrophages	[86]
Dermatitis	IL-1, TNF-α, IFN-γ	EC	[87]
Periapical periodontitis	IL-1β, IL-6, TNF-α, IFN-γ	Epithelial cells, EC, fibroblasts, macrophages, PMNL	[88]

TNF-α, Tumour necrosis factor-alpha; GM-CSF, granulocyte-macrophage colony-stimulating factor; TGF-β, transforming growth factor-beta; EC, endothelial cells; VSMC, vascular smooth muscle cells; PMNL, polymorphonuclear leucocytes.

produce NO *in vitro* [99–101]. All these data are suggestive of a similar role of iNOS activity in the human disease comparable to the animal models of diabetes. However, it has still to be shown whether inhibiting iNOS-derived NO in human patients will protect from tissue-destructive processes in RA, MS and type-1 diabetes.

iNOS expression has also been found in chronically inflammatory diseases of the airways, the vessels, the bowels, the kidney, the heart, the skin and the apex of teeth (Table 2). In these various diseases iNOS immunoreactivity has sometimes been localized to macrophages, but in most cases is found associated with epithelial cells around inflammatory foci. The role of epithelial iNOS activity is not really understood. The NO produced may either serve to limit bacterial invasion or may serve to limit local immune reactions and concomitant tissue destruction during Th1 immune responses. Local cytokine expression or cytokine response profiles in the relevant diseases positive for iNOS expression invariably correlate with the presence of proinflammatory Th1-type reactivities (Table 2). Of note is the association with IL-8, a cytokine usually labelled as chemoattractant, which on the ground of being a key inductor for iNOS expression in human keratinocytes [83] appears to exert proinflammatory activity in human diseases. Due to the close association of Th1-type cytokines and iNOS expression in human diseases, it could be argued that iNOS expression is an epiphenomenon of inflammatory diseases only. In this case iNOS expression would serve as an excellent marker for Th1 reactivity or imbalance. Future diagnostic evaluation may then use this one marker instead of measuring relative expressional levels of numerous cytokines.

iNOS EXPRESSION IN OTHER HUMAN DISORDERS

iNOS expression has been found in a variety of other human disorders (Table 3). However, courses of these diseases and cellular iNOS expression differ considerably.

iNOS and chronic neurodegeneration

iNOS protein was found post-mortem in the brains of patients with Alzheimer's and Parkinson's diseases [102–104]. Although neurones are highly susceptible to the cytotoxic action of NO, it is not yet clear whether iNOS expression accompanies late stages of disease or whether iNOS activity contributes to the course of these diseases.

iNOS and ischaemic events

As mentioned above, the human iNOS promoter contains a hypoxia-responsive element. Therefore it is not surprising that iNOS protein has been detected in cardiac myocytes and in infiltrating macrophages of patients several days after myocardial infarction [79,80,105]. In a rabbit heart infarction model, administration of specific NOS inhibitors significantly improved ventricular performance and increased myocardial blood flow in the surviving myocardium [117], suggesting tissue-destructive effects of NO production via iNOS. Moreover, after cardiac transplantation iNOS protein expression in myocytes and VSMC appears to correlate with contractile dysfunction [111].

Until now no data have been available concerning iNOS expression in human stroke. Data on animal stroke models imply that iNOS is expressed in areas of infarcted or injured brain. After

Table 3. Inducible nitric oxide synthase (iNOS) expression in human neurodegenerative diseases and heart infarction, during tumour development, after transplantation, during prostheses failure and myositis

Disorder	Localization of iNOS	References
Alzheimer's disease	Neurofibrillary tangle-bearing neurones	[102]
	Astrocytes	[103]
Parkinson's disease	Glial cells in the substantia nigra	[104]
Heart infarction	Myocytes	[79–81]
	Macrophages, myocytes	[105]
Tumours		
Brain	Vasculature, tumour cells	[106]
Breast	Macrophages, EC, some myoepithelial cells	[107]
	Tumour cells, EC, stroma	[108]
Lung	Tumour cells, macrophages	[109]
Colon	Mononuclear cells, EC, tumour epithelium	[110]
Heart transplantation/rejection	Myocytes, VSMC	[111]
Obliterative bronchiolitis	PMNL, macrophages, alveolar + airway epithelium, EC	[112]
Prostheses failure	Macrophages, EC	[113]
	Macrophages	[114]
	Macrophages, synovial lining cells, fibroblasts, VSMC	[115]
Inclusion-body myositis	Vacuolated muscle fibres, macrophages	[116]

EC, Endothelial cells; VSMC, vascular smooth muscle cells; PMNL, polymorphonuclear leucocytes.

focal cerebral ischaemia proinflammatory cytokines such as TNF-α, IL-1, and IL-6 are expressed, and neuronal tissue damage continues for days after the ischaemic event. Experimentally induced focal cerebral ischaemia in rats resulted in iNOS protein expression in neutrophils or in vascular cells 12–48 h after the ischaemic event, depending on the stroke model. In mutant mice deficient in nNOS or iNOS, infarct volume and neurological deficits were significantly smaller than those in normal mice. In contrast, eNOS knockout mice developed larger infarct volumes than the corresponding wild-type strains (for review see [118]). This proves the complexity of the NO-mediated effects, especially in brain, where high enzyme activities of eNOS (to maintain cerebral blood flow) and nNOS (to perform signalling functions) are normally present. Thus, low-output NO production by endothelial cells appears to promote regional cerebral blood flow during ischaemia, while neuronal NO production via nNOS and later long-lasting high-output production of NO via iNOS by inflammatory cells may be neurotoxic.

iNOS and tumours

The role of NO during tumour development also reveals a complex picture. High-output NO production by infiltrating macrophages can induce tumour cell cytostasis and/or cytotoxicity. iNOS protein has been detected in the vasculature, infiltrating macrophages, and tumour cells of human brain, breast, lung, and colon tumours (see Table 3). In rats, i.p. injection of colon adenocarcinoma cells was accompanied by a decrease in concanavalin A (Con A)-induced splenic T lymphocyte proliferation correlating with an increased NO production by splenic macrophages [119]. Low-output NO production within the tumour may increase tumour blood flow and promote angiogenesis [120]. In addition, NO produced by the tumour itself may inhibit proliferation or induce apoptosis of T lymphocytes, which would explain the suppression of host immune functions often observed to accompany tumour growth.

iNOS and transplantation/implantation

In recipients of myocardial allografts, iNOS protein was found in myocytes and VSMC. However, although iNOS mRNA was detected in all patients at some stage, this was episodic and occurred most frequently during the first 180 days after transplantation. It was postulated that iNOS expression and contractile dysfunction are causally related [111]. iNOS was also detected in various cell types involved in foreign body inflammatory reactions found around loosened joint replacement implants [113–115]. iNOS activity of activated and prosthetic wear debris-laden macrophages is likely to be noxious and may thus contribute to early prosthesis failure.

iNOS expression after noxious insults

iNOS mRNA was found in all skin biopsies taken from healthy volunteers treated with a single dose of either UV-A or UV-B, in contrast to untreated controls. iNOS protein was labelled in a band-like pattern confined to the highly proliferative basal layer of keratinocytes only [84]. iNOS expression was found to be maximal at 24 h, while 72 h post-irradiation none of the biopsies showed iNOS-specific signals, thus closely following the kinetics of erythema formation after sunburn which peaks at 24 h and lasts for 2 days. It appears as if iNOS activity is the cause of the observed long-lasting increase in local blood flow as well as erythema and oedema formation after prolonged sunlight exposure. *In vitro*, endogenous NO production via iNOS or exogenously added NO protected human dermal endothelial cells from UV-A-induced DNA fragmentation and subsequent apoptosis [121]. Thus, increased NO production may help to reduce UV-induced damage as a first indication of a protective role for iNOS expression in human skin. In animal models, NO plays a positive role in wound healing. Mice deficient in iNOS exhibit impaired wound healing, and iNOS gene transfer reverses the impaired healing of excisional wounds (see [122]).

The inflammatory response after traumatic brain injury (TBI)

includes cytokine production, leucocyte infiltration, and microglial activation. In rats, 24 h and 48 h after experimentally induced TBI a marked peritrauma cerebrovascular iNOS protein expression was found predominantly in infiltrating neutrophils [123]. This suggests a role for iNOS activity in cerebrovascular disturbances and secondary brain injury after head trauma. Although data are not yet available, iNOS expression after TBI in man is most likely.

CONCLUDING REMARKS

Studies performed in rodents mostly imply that iNOS activity plays a detrimental role in experimental autoimmune or chronically inflammatory processes as well as in some other diseases. However, experimentally induced disorders are often constructed to show a maximal effect in a relatively short period of time. In addition, in many rodent strains Th1-associated reactions are favoured, whereas in humans an individually regulated balance between Th1- and Th2-mediated immune reactions is found. Thus, the question arises whether we can infer from animal (rodent) studies a role of iNOS in human diseases. It is crucial that we learn about the relative timing of disease onset and iNOS expression, especially as clinically overt disease often represents a relatively late-stage process. Another major problem in understanding the role of iNOS in human diseases is our lack of knowledge whether enzyme expression *in vivo* results in high- or low-output NO production. Although the human iNOS gene is more tightly regulated than the respective rodent gene (see first section), this does not necessarily imply low-output NO production in humans in all cases. Few reports only demonstrate high-output NO production by human cells *in vitro*, among these macrophages isolated from an MS lesion, hepatocytes, dermal endothelial cells, and polymorphonuclear leucocytes (PMNL) from periapical periodontitis patients, all producing high NO concentrations similar to activated rodent cells [64,124–126]. Thus, at least *in vitro*, human cells are indeed capable of high-output NO synthesis. Moreover, human cells appear to be more resistant to NO-mediated effects [127]. This may be due to a more effective DNA repair or to a higher capacity to induce protective mechanisms, e.g. expression of heat shock proteins (hsp). With rat islet cells over-expression of hsp70 has been shown to confer resistance against NO [128], and suppression of hsp70 by antisense RNA transfection of a human islet cell line confers susceptibility to NO-mediated cytotoxicity [129].

In summary, many data on human disorders with predominant proinflammatory Th1 reactions involving activated macrophages and neutrophils point to a destructive activity of iNOS contributing to local tissue damage, but as pointed out earlier, iNOS expression is often found predominantly in cells of epithelial origin (see Table 2). We do not yet know the reason for this, but it may well be that in these cases NO serves as a protective agent limiting bacterial invasion or down-regulating local inflammatory reactions by inducing cytostasis and/or apoptosis in infiltrating immune cells like macrophages, neutrophils or T cells (for review see [1]). NO is known to affect gene regulation, e.g. via inhibition of NF-κB activation or by inhibiting the DNA-binding of NF-κB, AP-1 [1], and zinc finger transcription factors like Sp1 [130], thereby limiting Th1 reactions. Gene regulatory activities, instead of direct cytotoxic actions of iNOS-derived NO, may be of higher relevance in human diseases compared with experimentally induced rodent diseases (Fig. 2). Data are accumulating that NO not only induces but also inhibits programmed cell death induced by TNF-α or anti-CD95 (Fas/APO-1) [131,132]. We recently found that iNOS activity completely protects from UV-induced apoptosis in dermal endothelial cells [121]. A role for NO in protecting against adverse effects by reactive oxygen intermediates has also been postulated.

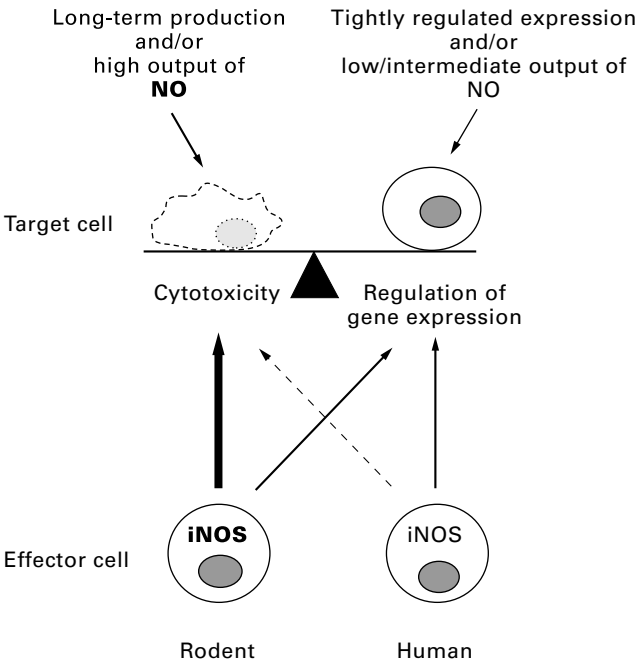

Fig. 2. Current data suggest that inducible nitric oxide synthase (iNOS) expression and NO production in humans are much more tightly regulated than in rodents. In addition, human cells appear to be less susceptible to NO than rodent cells. Thus, in humans NO-mediated gene regulatory effects may be of more relevance than cytotoxic effects.

In conclusion, a large amount of data concerning iNOS expression in a variety of human diseases has accumulated, but we are still far from understanding the precise role of iNOS activity in most of these diseases.

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