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Response of endothelial and mesangial cells to pro-inflammatory stimuli. Regulation and activation of hemopexin and alkaline phosphatase

Kapojos, Jola Jovita

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Document Version

Publisher's PDF, also known as Version of record

Publication date:

2003

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Kapojos, J. J. (2003). *Response of endothelial and mesangial cells to pro-inflammatory stimuli. Regulation and activation of hemopexin and alkaline phosphatase.* s.n.

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In the present thesis interactions of activated endothelium or mesangium cells with proinflammatory cytokines like $TNF\alpha$, IL-6 or toxic agents like LPS were studied. These cells, in particular their potential to locally upregulate detoxifying enzymes like alkaline phosphatase (AP) was examined in experimental animals in vivo as well as in cell cultures of endothelial or mesangial cells. A second aspect in the present thesis concerns the potential of these stimulated cells in vitro to release hemopexin (Hx) or to turnover non-active Hx into its active isoform.

The rationale of the first experiments comes from observations showing transient fluctuations of AP after challenge with *E. coli* bacteria or LPS as an LPS detoxifying host defence principle^{1,2}. As the glomerular microvasculature of the kidney has its own specific defence machinery, consisting of ecto-nucleotidases, inhibitors of inflammatory cytokines, proteinase inhibitors or oxygen radical scavengers³, the question emerged whether also such an inducible anti endotoxin response could be shown in glomeruli of the rat in vivo.

The background of the study on Hx in relation to endothelium and mesangium stems from clinical observations in subjects with corticosteroid responsive nephrotic syndrome (CRNS) in relapse in combination with their characteristic glomerular alterations. Up to now, the pathogenesis and the etiology of this disorder is obscure; it is believed that cellular immune aberrations for instance leading to an enhanced level of $TNF\alpha$ in the circulation^{4,6} and certain proteinuria inducing molecules present in plasma (for instance activated Hx) may play a role in the pathogenesis of CRNS⁷. Patients with CRNS in relapse show enhanced Hx activity as compared with subjects in remission or healthy individuals (see p. 112-113). Moreover, as is mentioned in chapter 1, intrarenal infusion of Hx into rats induces proteinuria in combination with the characteristic CRNS like glomerular lesion in these animals⁸.

Therefore it was reasoned that the inactive form of Hx (Hxi), which is kept in its inactive form under normal circumstances by various protease inhibitors or extracellular nucleotides like ATP (chapter 3), may be reactivated in CRNS. Thus, cytokine-stimulated endothelial or mesangial cells, with enhanced ecto-apyrase due to increased $TNF\alpha$, may be responsible for this reactivation in subjects with CRNS in relapse. Such a mechanism may explain enhanced peripheral vascular permeability

in these patients⁹, next to the responsiveness of the vessel wall to the question whether it can be influenced by Hx. This is devoted to alterations in Hx activity with prednisolone, since it is known that conversion of non-active Hxi into active Hx in vitro. Finally the question whether intrarenal Hx infusion has given rise to

The work described in this chapter on endothelial cells follows the same lines.

The first study was performed in HUVECs after challenge with LPS. As mentioned above, AP activity is a marker of this bacterial product on endothelial cells followed by immunocytochemistry and cytochemistry. Also the effect of LPS on Hx activity was tested in vitro as test system for the administration of LPS in vivo on glomerular AP. Further studies are devoted to detoxify LPS. Since Hx is known to upregulate AP activity in vivo represents a local defence mechanism (chapter 2).

The next set of experiments was performed on endothelial and mesangial cells. The effect of activated isoform in vitro on glomerular permeability was tested. It is high in the healthy control and in the cultures of HUVECs of the vessel wall, in combination with the cultures of HUVECs

in these patients⁹, next to their enhanced glomerular permeability. The corticosteroid responsiveness of the nephrotic syndrome associated with Hx activation has led to the question whether upregulation of ecto-apyrase in endothelial and mesangial cells can be influenced by prednisolone. Therefore a part of the present thesis has been devoted to alterations of the expression of ecto-apyrase of these cells after treatment with prednisolone, since this ecto-enzyme has been shown to mediate the conversion of non-active Hxi into its enzymatic form Hxa in endothelial and mesangial cells in vitro. Finally the question as to the possibility that Hxa synthesis may also occur intrarenally has given rise to the in vitro experiments described in chapter 5.

The work described in this thesis explores the role of mesangial and endothelial cells following proinflammatory stimulation in vitro and in vivo.

The first study was designed to investigate induction of glomerular AP of rats after challenge with LPS or E. coli bacteria in vivo, using histochemical methods. As mentioned above, AP is able to dephosphorylate LPS, thereby attenuating the toxicity of this bacterial product. In addition, AP was also studied in mesangial and endothelial cells following stimulation by cytokines or LPS in vitro by immunostaining and cytochemistry. Also the mRNA for AP was studied following challenge with these agents in vitro as tested using molecular biological methods. The results show that administration of LPS or E. coli in vivo is able to induce local upregulation of glomerular AP. Furthermore, it was shown that this glomerular ecto-enzyme is able to detoxify LPS. Since also cultured endothelium and mesangial cells are able to upregulate AP activity and expression, it is likely that the glomerular upregulation in vivo represents a local event rather than influx of circulating AP in the glomerular tuft (chapter 2).

The next set of experiments was designed to explore the role of stimulated endothelial and mesangial cells regarding the conversion of inactivated Hx into its activated isoform in vitro. Activated Hx is able to induce increased vascular and glomerular permeability, whereas circulating levels of inactive Hx are supposed to be high in the healthy condition; thus to explain local induction of increased permeability of the vessel wall, in situ activation of Hx may be quite relevant. The results show that the cultures of HUVEC or human mesangial cells stimulated with LPS or TNF α

appeared to be able to turn over inactive Hx into its enzymatic isoform (Hxa). This effect is mediated by ecto-apyrase, although a contribution of ecto-AP can not be excluded. If these data reflect the *in vivo* situation, they illustrate the link between a cellular immunologic event (like pro inflammatory cytokine production) on the one hand, and enhanced activity of a potentially proteinuric factor on the other hand (chapter 3).

The subsequent study was designed to explore the sensitivity of mesangial or endothelial ecto-apyrase for corticosteroids, since this ecto-enzyme plays a major role in the conversion of inactive Hx into activated Hx. Supplementation of prednisolone to stimulated cells of the culture systems studied, resulted in down regulation of ecto-apyrase; low ecto-apyrase activity decreases the potential conversion of inactivated Hx into its active isoform, in other words these data suggest that the turnover of Hxi into Hxa *in vitro* is corticosteroid dependent. As soluble apyrase activity measured by a biochemical assay is also inhibitable by corticosteroids whereas the mRNA signal for apyrase is not reduced in prednisolone-treated cells, it is likely that the prednisolone effect observed is non-genomic i.e. the effect is exerted directly towards (membrane) proteins rather than through transcription pathways. This notion is supported by the observation that treatment of the cells with mifepristone does not abolish the prednisolone effect. [Mifepristone is a corticosteroid receptor antagonist known to act through transcription pathways] (chapter 4).

The next experiments were designed to investigate the possibility that human mesangial cells *in vitro* are able to produce Hx following an appropriate proinflammatory stimulation by cytokines like $\text{TNF}\alpha$, IL-6 or IL-1. It was shown that mesangial cells are able to produce and release Hx *in vitro* after stimulation with $\text{TNF}\alpha$, and that this production can be inhibited by prednisolone. It was also demonstrated that the mRNA signal for Hx is diminished in these cells following treatment with prednisolone. This observation opens the possibility that also *in vivo* local stimulation of mesangial cells by $\text{TNF}\alpha$ may give rise to intraglomerular release of Hx. Thus, in subjects with CRNS not only circulating Hx may be activated due to enhanced cytokine levels in the circulation, but cytokines like $\text{TNF}\alpha$ may also be able to cause intrarenal release of this proteinuria-inducing mediator (chapter 5).

Alkaline phosphatase

Constitutive expression in man, as is the case with expression is less inducible glomerular expression most likely reflects a of AP after stimulation this enzyme may also glomerular tuft³. Since and ADP (and these also be considered endothelium *in vivo* upregulated in endo corticosteroid dependent ecto-enzymes in end now.

One function turnover Hxi into reactivation mechanism Further experimental stimulated endothelium corticosteroids (un that at this moment be drawn.

Hx, vascular permeability

As observed by v like upper respiratory