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Hypothetical molecular mechanisms by which local iron overload facilitates the development of venous leg ulcers and multiple sclerosis lesions

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Summary This paper presents a hypothetical model of role for iron in the development of venous leg ulcers and multiple sclerosis. Elevated concentrations of iron were found in the skin affected by venous hypertension and also in the areas of brain with multiple sclerosis lesions. Individuals with hemochromatosis gene (HFE) mutations: C282Y and H63D, which result in a less efficient transport of iron by macrophages, are characterized by an increased risk for venous leg ulcer and multiple sclerosis. Multiple sclerosis is a T cell-mediated disease, and T cells probably participate in the development of venous ulcers. This deleterious role of ferric ions could be related to the regulation of T cell proliferation and apoptosis. Under normal conditions excessive accumulation of T cells cannot take place, because nitric oxide and interferon-gamma drive these cells toward apoptosis. However, in tissues with a high concentration of iron, T lymphocytes proliferate instead of undergoing apoptosis. This is possible due to the internalization of the INF-γR2 chain of the interferon-gamma receptor, the downregulation of inducible nitric oxide synthase expression in macrophages and the inactivation of the active site of caspases. Yet, it should be emphasized that this hypothesis does not claim for the increased concentration of iron as a direct causal factor for the development of venous ulcerations or multiple sclerosis, but rather, iron is a factor that modulates and exaggerates the autoimmune process. Iron chelators, administered systemically or locally, should potentially exhibit therapeutic and prophylactic activity against venous leg ulcers and multiple sclerosis.

In spite of a high prevalence and significant morbidity of venous leg ulcers, mechanisms leading from venous hypertension to the development of ulceration have not been yet determined. Evidence suggests that factors at cellular and molecular levels may be important; relevant studies in this field, however, are still missing [1,2]. Similarly, multiple sclerosis is a chronic, immune-mediated, demyelinating disease of the central nervous system of as yet unknown aetiology, although it is suspected that this disorder is the result of interplay between environmental factors and susceptible genes [3].

Yet, the deposition of iron has been recognized as a potential pathophysiologic factor responsible

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for the development of both pathologies. Elevated concentrations of iron were found in the skin affected by venous hypertension and also in the areas of brain with multiple sclerosis lesions [1]. The haemosiderosis of the dermis is the manifestation of failure of metabolism and transport of ferric ions in the skin, while grey matter hypointensity on T2-weighted magnetic resonance imaging scans, reflecting the iron deposition, has been described in multiple sclerosis [4–6]. In the dermis of a venous leg ulcer patient this local iron overload is caused primarily by extravasation of erythrocytes, which is a result of venous hypertension that breaks junctions between endotheliocytes; in multiple sclerosis it is thought to result from an interruption of the blood-brain barrier, however, the exact mechanism leading to this phenomenon remains unknown, and probably it differs from that of a chronic venous insufficiency. Moreover, it has been found that the urine excretion of iron in venous leg ulcer and multiple sclerosis patients is increased, thus indicating a dysmetabolism of this metal [1,2,7]. Although a role for iron in the pathogenesis of both above-mentioned diseases with a potential autoimmune background remains elusive, individuals with the hemochromatosis gene (HFE) mutations: C282Y and H63D, which result in a less efficient transport of iron by macrophages, are characterized by an increased risk for venous leg ulcer development [1,2,8–10]. Yet, in parallel, while a significant correlation between the HFE polymorphism and multiple sclerosis susceptibility has not yet been found, there are data indicating that mutations of this gene may play a role in the predisposition to this disease [11,12].

Traditionally, because ferric ions are the most important inducers of reactive oxygen species, it is speculated that deleterious role for iron is related to the generation of aggressive free radicals and iron-triggered over-expression of matrix metalloproteinases, [1,2,9,13–15]. Data from several studies indicate that free radicals may participate in the pathogenesis of venous ulcerations and multiple sclerosis, and ferric ions have been implicated as a catalyst leading to their formation [13]. However, despite this increased attention and awareness, our knowledge of iron metabolism at the cellular and molecular levels is still limited [16]. Importantly, alternative explanations of the role for iron in the development of venous leg ulcers and multiple sclerosis are also possible. Potentially, this could be related to the regulation of the T cell proliferation and apoptosis. This mechanism has already been suggested by Grant et al., [18] who investigated the influence of iron-deficiency on the development of murine experimental autoimmune encephalomyelitis, which is one of the most useful models of multiple sclerosis, and also by Leung et al., [19] who investigated the influence of iron chelators on functions and proliferation of T cells with the Th1 phenotype. Yet, the exact molecular pathways responsible for this iron-triggered hyper-reactivity of the T cells remain unclear. Therefore, the goal of our paper is to present these potential mechanisms.

A consensus of opinion has suggested that multiple sclerosis is a T cell-mediated autoimmune disease [3,17,20]. There are also some data indicating that T cells contribute to the clinical course of venous leg ulcers [1,21,22]. The idea that iron concentration determines the fate of a T cell could be of critical interest in the understanding of these autoimmune disorders. T cells are susceptible to many factors that can determine their fate (proliferation or apoptosis). Among these regulators, interferon-gamma (INF-γ) and nitric oxide (NO) appear to be of great importance. Interestingly, both regulators could be influenced by a local concentration of iron.

INF-γ that is secreted by T cells and macrophages is one of the principal regulators of T cell proliferation and apoptosis. Usually, INF-γ triggers the apoptosis of a T cell. INF-γ exerts its actions though the receptor INF-γR, which consists of two chains: INF-γR1 and INF-γR2. The fate of a target T cell primarily depends on the expression of these two subunits. INF-γ triggers apoptosis of the cells that express both subunits. On the contrary, T cells that express only INF-γR1 become resistant to antiproliferative and proapoptotic action of INF-γ, and INF-γ favours their proliferation and differentiation [23,24]. In human T cells downregulation of the INF-γR2 occurs mainly through the internalization of this protein. This internalization is dependent on insulin-like growth factor-1 (IGF-1) [25]. In the skin, IGF-1 is produced primarily by macrophages, and this production is decreased by INF-γ [26]. There is a negative feedback loop between IGF-1 and IFN-γ, and INF-γ exhibits the protective function through decreasing the IGF-1-dependent internalization of INF-γR2. It could be hypothesized that similar interactions between INF-γ and IGF-1 may also occur in the central nervous system. However, the internalization of the INF-γR2 chain is also enhanced by ferric ions [27]. Thus, iron can determine the fate of T cells exposed to INF-γ and induces their refractoriness to the INF-γ-mediated apoptosis.

NO is the other important mediator of T cell apoptosis. In the skin the majority of NO is produced by activated macrophages, and the macrophage production of NO depends primarily on the expression of inducible nitric oxide synthase

(iNOS). This expression of iNOS can be enhanced by INF-γ. On the other hand, INF-γ that is produced by T cells exhibits antiproliferative action on macrophages, though some observations suggest that Granulocyte Macrophage-Colony Stimulating Factor, which is simultaneously released by T cells, is able to protect macrophages from the antiproliferative action of INF-γ [23]. Nevertheless, there is an interplay between NO and INF-γ that results in the control of unrestrained proliferation of T cells and macrophages [28,29]. In the central nervous system, apart from iNOS expressed by macrophages, NO is also produced by neuronal nitric oxide synthase (nNOS) and endothelial nitric oxide synthase (eNOS) [30]. It has been found that iron inhibits the macrophage expression of iNOS [31], perhaps disturbing the NO-dependent negative feedback loop between macrophages and T cells. Moreover, in particular settings, NO either induces apoptosis, or protects a cell from programmed death. This NO-mediated apoptosis is related to activation of caspases. Caspases are the family of proapoptotic enzymes. However, apart from the activation of caspases, NO can also cause their inactivation through the *S*-nitrosylation of cysteine within the active site of an enzyme. This NO-mediated *S*-nitrosylation requires giving up an electron to favour the formation of a nitrosonium-like species. Iron appears to be an important electron acceptor in this process [32,33]. The consequences of NO exposure depend on the intracellular content of iron. Cells with a great amount of intracellular iron, for example endotheliocytes, are resistant to NO-mediated apoptosis [28]. Thus, T cells usually develop apoptosis after NO exposure, but may become resistant to caspase-mediated apoptosis in the settings characterized by a high iron concentration. Interestingly, in the experimental settings, iNOS inhibition resulted in the prevention of clinical signs of autoimmune encephalomyelitis, an animal model of multiple sclerosis [33]. However, mice genetically deficient for iNOS were shown to be susceptible to autoimmune encephalomyelitis [29], indicating that perhaps NO is not crucial for the development of multiple sclerosis. Yet, it should be remembered that this animal model may differ from multiple sclerosis in humans.

Thus, it could be hypothesized that deleterious role for iron in the development of venous leg ulcers and multiple sclerosis lesions is potentially related to the abundant accumulation of T cells with the Th1 phenotype in the pericapillary interstitium. Under normal conditions such accumulation cannot take place, because NO and INF-γ drive T cells toward apoptosis. However, in the tissues with a high concentration of ferric ions these cells proliferate, instead of undergoing programmed death, and can mediate injury to the skin or nervous tissue. Hopefully, this hypothesis could provide a useful framework for future research. Yet, it should be emphasized that this hypothesis does not claim for the increased concentration of iron (for example — following blood transfusion, or in a thalassemic patient) as a direct causal factor for the development of venous ulcerations or multiple sclerosis. Likely, it is not iron that triggers these pathologies, but rather, iron is a factor that modulates autoimmune process, which is potentially self-limiting, but in the context of a high local concentration of ferric ions it exaggerates and finally leads to an overt disease. It should be mentioned, however, that patients suffering from sickle cell disease and thalassemia are characterized by a high risk for development of leg ulcerations. The exact pathomechanism of development of these ulcers remains unclear. It is thought to be a result of the combination of local ischemia, venous insufficiency and increased susceptibility for bacterial infection [34—36]. A role for iron-overload in the pathogenesis of these ulcers, however, cannot be completely ruled out. On the contrary, thalassemia and sickle cell disease are not associated with an increased risk for multiple sclerosis. In addition, iron-overloaded mice do not develop more severe autoimmune encephalomyelitis than normal-iron animals [33].

There are several strategies that could reduce this noxious local iron overload. Although potentially locally acting iron-reducing agents should be more efficient, systemic administration of a chelating agent might be more successful and easier. Recently it has been revealed by Mitchell et al. [37] that orally-given deferiprone, an iron chelator, had a positive clinical effect in an animal model of multiple sclerosis. Interestingly, this iron chelator not only suppressed autoimmune encephalomyelitis in experimental animals, but also it inhibited the T-cell function. For a hypothetical treatment of multiple sclerosis or venous ulcers, iron chelators could be administered in a much lower doses than those recommended for the management of thalassemia. Local administration of iron chelators could be the other option for venous ulcer patients. Subcutaneously injected iron chelator — deferoxamine mesylate — appeared to be safe and efficient in the management of skin hyperpigmentation following venous sclerotherapy [38]. Moreover, restraining the excessive erythrocyte extravasation, for example by strengthening the intercellular junctions in the endothelium, could be the other therapeutic option. However, currently, there are no drugs exhibiting this action.

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