European Journal of Immunology

HIGHLIGHTS

Basic

REVIEW

The macrophage migration inhibitory factor/CD74 axis in traumatic spinal cord injury: lessons learned from animal and human studies

Serina Rubio, Veerle Somers and Judith Fraussen

Department of Immunology and Infection, Biomedical Research Institute, Hasselt University, Hasselt 3500, Belgium

1st time author
Read more about EJI's first-time authors

Traumatic spinal cord injury (SCI) is a severe condition leading to long-term impairment of motor, sensory, and autonomic functions. Following the initial injury, a series of additional events is initiated further damaging the spinal cord. During this secondary injury phase, both an inflammatory and immune modulatory response are triggered that have damaging and anti-inflammatory properties, respectively. The proinflammatory cytokine macrophage migration inhibitory factor (MIF) and its receptor CD74 have been extensively studied in traumatic SCI. MIF expression is increased in spinal cord tissue after experimental SCI, mainly in astrocytes and microglia, as well as in the plasma of SCI patients. Functionally, MIF and CD74 were shown to regulate astrocyte viability, proliferation and cholesterol metabolism, microglia migration, and neuronal viability. Moreover, inhibition of the MIF/CD74 axis improved the functional recovery of SCI animals. We provide a detailed overview of studies analyzing the role of MIF and CD74 in traumatic SCI. We describe results from animal studies, using rat and mouse models for SCI, and human studies. Furthermore, we propose a new path for investigation, focused on B cells, that might lead to a better understanding of how MIF and CD74 contribute to the secondary injury cascade following traumatic SCI.

Keywords: Animal studies · B cells · Human studies · MIF/CD74 axis · Traumatic spinal cord injury

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Introduction

Traumatic spinal cord injury (SCI) is a severe condition that leads to long-term impairment of motor, sensory, and autonomic functions [1]. Every year, between 250,000 and 500,000 patients worldwide suffer from SCI resulting from motor vehicle accidents, falls, violence, and other causes [1], [2]. Traumatic SCI is more common in males, especially during young adulthood (20–29 years) and in elderly individuals (>70 years) [1]. However, the average age at injury has increased from 29 years during the 1970s to 43 years because of etiologic and demographic changes in SCI [2]. As this condition may cause long-term disabilities, the costs that are directly and indirectly related to the injury are very high and cause a substantial economic burden on society [2], [3]. Treatment options may include stabilization and decompression surgery, nonselective immunosuppression, rehabilitation therapy, and treatments for secondary complications [4], [5]. However, these therapeutic options are limited and no standardized protocols are available.

Correspondence: Dr. Judith Fraussen
e-mail: Judith.fraussen@uhasselt.be

After patients have suffered from an SCI, their motor, sensory, and anorectal functions are examined using the International Standards for Neurological Classification of Spinal Cord Injury (ISNCSCI) exam, which includes the American Spinal Injury Association (ASIA) Impairment Scale (AIS) [6]. Five specific muscle groups in both the upper and lower extremities are evaluated for motor strength to grade motor functions. Sensory functions are evaluated by examining 28 specific dermatomes for light touch and pinprick sensation. The examination is finalized by evaluating the external anal sphincter for voluntary motor contraction and deep anal pressure [6]. Eventually, SCI patients are categorized into five AIS grades. Grade A is the most severe one, including patients who have a complete injury, with no motor or sensory function in the sacral segments S4–S5. Grade B patients have a motor complete and sensory incomplete injury, with preserved sensory functions but no motor function below the neurological level or in the sacral segments. Other patients are classified as motor incomplete, namely grade C when less than half of the key muscles below the neurological level have a motor score $\geq$3, or grade D otherwise. Lastly, grade E corresponds with a return to normal sensory and motor functions [6].

Traumatic SCI consists of a primary and a secondary injury phase [7], [8]. An external force causes disruption and dislocation of the vertebral column and injures the spinal cord due to contusion, transection, or compression. Consequently, damage is inflicted on local neurons and glial cells [9]. The blood-spinal cord barrier is also disrupted, leading to hemorrhages, edema, and ischemia [10], [11]. A series of additional events is initiated during a secondary injury phase that further damages the spinal cord. This secondary injury phase can last up to months after the primary injury and is therefore subdivided into three, highly overlapping, phases: acute, subacute, and chronic phases. In the acute phase, hemorrhages, edema, and ischemia persist due to the disrupted vasculature [10]. This is also accompanied by an enormous influx of immune cells and the production of cytokines [12–14]. These immune cells can be locally present in the spinal cord, such as microglia, or infiltrate from the periphery, such as macrophages, B cells, and T cells [12], [13]. Various proinflammatory cytokines, such as TNF-α and IL-1β, which are released by these immune cells, further aggravate tissue injury [14]. Neuronal and glial cell death is also apparent. In the subacute phase, the immune cells clear cellular and myelin debris, and secrete cytokines to facilitate repair. Simultaneously, they release cytotoxic by-products, such as free radicals, causing even more damage, as reviewed in [7], [8]. Neuronal damage and cell death also lead to excessive glutamate release and subsequent excitotoxicity [15–19]. In order to limit the injury and prevent further spreading, astrocytes start to proliferate and produce extracellular matrix proteins that will eventually form the glial scar that surrounds the cystic cavity in the chronic phase [9], [10]. Despite its protective nature, the glial scar also creates a physical and chemical barrier that hinders the already limiting repair and regeneration processes [20], [21].

A thorough investigation of all the inflammatory players in this secondary injury phase, such as immune cells and cytokines, is needed to understand the complexity of traumatic SCI and define their role in both the reparative and damaging processes that are initiated. Macrophage migration inhibitory factor (MIF), a proinflammatory and pleiotropic cytokine, has been a topic of research in traumatic SCI as it was shown to have effects on immune cells but also astrocytes, neurons, and epithelial cells of the choroid plexus in the CNS [22–26]. Its discovery dates back to 1966 when MIF was found to be produced by T cells and inhibited the migration of macrophages in vitro in the context of hypersensitivity [27]. To date, MIF has been studied in many different diseases, such as cancer, rheumatoid arthritis, Parkinson's disease, sepsis as well as traumatic SCI, proving its pleiotropic nature [28–31]. This review covers MIF and its receptor CD74 in traumatic SCI, and, more specifically, discusses the different animal and human studies that have focused on this cytokine-receptor complex in traumatic SCI.

MIF and CD74

MIF is a 12 kDa proinflammatory cytokine that binds to the receptor CD74 [32], [33]. Due to its homotrimer structure, three binding sites are created to bind to CD74, which also forms homotrimers [34]. Independently of CD74, MIF can also function as a tautomerase and nuclease [35], [36]. MIF is highly expressed in distinct cell types, such as lymphocytes, monocytes/macrophages, endothelial cells, epithelial cells, astrocytes, and many others [37]. CD74 is mainly expressed on antigen-presenting cells, such as dendritic cells, B cells, and macrophages, but under inflammatory conditions, CD74 is also expressed on other cell types (i.e. endothelial and epithelial cells) [37]. Although CD74 was first defined as a major histocompatibility complex (MHC) class II chaperone, some excess of CD74 is expressed on the cell surface as a receptor for MIF [38]. After MIF binds to CD74, the CD44 co-receptor is recruited and serves as a signal transducer [39]. Both CD74 and CD44 are activated via protein kinase A-mediated phosphorylation of their intracellular domains, leading to an interaction between CD44 and the protein tyrosine kinase Src, and subsequent phosphorylation and activation of Src [39]. Additionally, the tyrosine kinase Syk is phosphorylated and activated (Fig. 1) [40], [41]. These tyrosine kinases then activate the extracellular signal-regulated kinase (ERK)/mitogen-activated protein kinase (MAPK) signaling pathway and the phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt) signaling pathway [39–41]. Research on B cells revealed that the PI3K/Akt pathway also induces cleavage of the intracellular domain of CD74 (CD74-ICD) [41], [42]. This CD74-ICD translocates to the nucleus and induces NF-κB activity [42]. Besides CD74 and CD44, MIF has also been found to be a noncognate ligand for the CXC-motif chemokine receptors (CXCR)2, 4, and 7, and thus has chemokine-like properties [23], [43], [44]. MIF can directly bind to all three receptors;

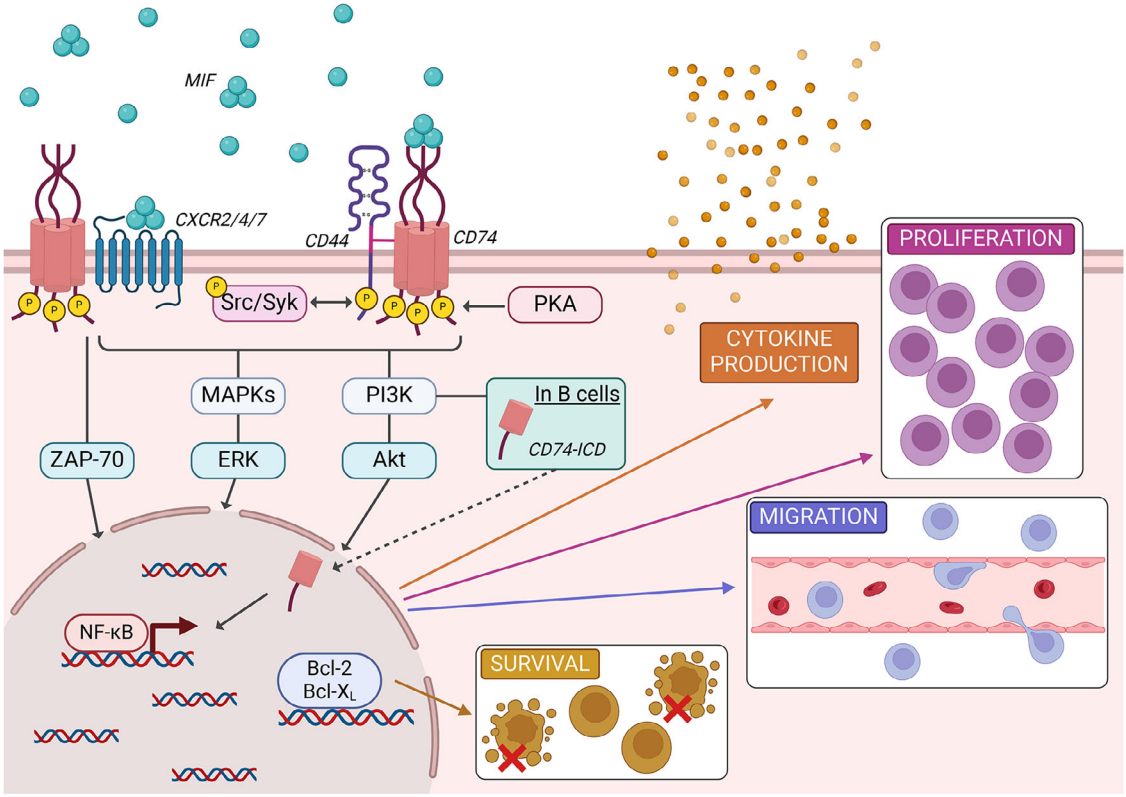

Figure 1. Schematic representation of the intracellular MIF/CD74 axis. Macrophage migration inhibitory factor (MIF) binds to the CD74 surface receptor, which leads to the recruitment of the CD44 co-receptor. CD74 and CD44 are phosphorylated and activated via protein kinase A (PKA). CD44 interacts with Src and/or Syk tyrosine kinases, which results in their phosphorylation and activation. MIF can also directly bind to the CXC-motif chemokine receptors CXCR2, CXCR4, or CXCR7; however, CD74 is needed for downstream signaling. Binding of MIF to its receptors leads to activation of the extracellular signal-regulated kinase (ERK)/mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt) pathways, as well as zeta-chain-associated protein kinase (ZAP-70) activation after binding to the chemokine receptors. The different downstream signaling pathways induce cell proliferation, migration, and survival by expression of anti-apoptotic factors (B cell lymphoma-2 (Bcl-2) and -extralarge (Bcl-X_L) and proinflammatory cytokine production. Additionally, as shown in B cells, the PI3K/Akt pathway induces the cleavage of the intracellular domain of CD74 (CD74-ICD). This CD74-ICD can translocate to the nucleus and activate NF-κB.

however, CD74 is needed to elicit downstream signaling [37]. Hence, CD74 can form complexes with all three chemokine receptors [43–45]. Whether CD44 is involved, is still unknown. MIF binding to the chemokine receptors also results in the activation of the ERK/MAPK and PI3K/Akt signaling pathways, as well as the activation of zeta-chain-associated protein kinase (ZAP)-70, which is a member of the Syk tyrosine kinase family (Fig. 1) [23], [44], [45]. Eventually, the different MIF-induced pathways promote cell proliferation and cell entry into the S-phase [40], [41], [46], [47]. Cell survival is promoted through the expression of anti-apoptotic and survival factors, such as B cell lymphoma-2 (Bcl-2) and -extralarge (Bcl-X_L), and the repression of cell death receptors [40], [41], [46], [48]. Furthermore, MIF-receptor binding leads to cell chemotaxis and migration, because of facilitated calcium influx and actin cytoskeletal reorganization [23], [44]. Finally, MIF induces the production of proinflammatory cytokines, such as TNF and IL-6 (Fig. 1) [46].

Although MIF mostly exerts proinflammatory effects, it has also been implicated in reparative processes, together with CD74. For example, MIF and CD74 have been found to promote wound healing in inflammatory bowel disease [49]. After lung injury, MIF signaling via CD74 resulted in the proliferation and differentiation of progenitor cells into lung epithelial-like cells, which may play a role in restoring the alveolar barrier [50]. Interestingly, MIF has also been found to promote peripheral nerve regeneration and Schwann cell survival and induce an inflammatory state in Schwann cells through CD74, which is beneficial for axonal regrowth [51], [52]. These studies question the black-and-white thinking that MIF is either a pro- or anti-inflammatory cytokine but instead suggest that MIF might balance between contributing to damage as well as repairing injury.

The MIF/CD74 axis in traumatic SCI animal studies

Rats and mice are the two most widely used animal species for traumatic SCI research. In both, the injury and secondary outcomes closely relate to the human situation [53]. Furthermore, different injury models exist, for which contusion, compression,

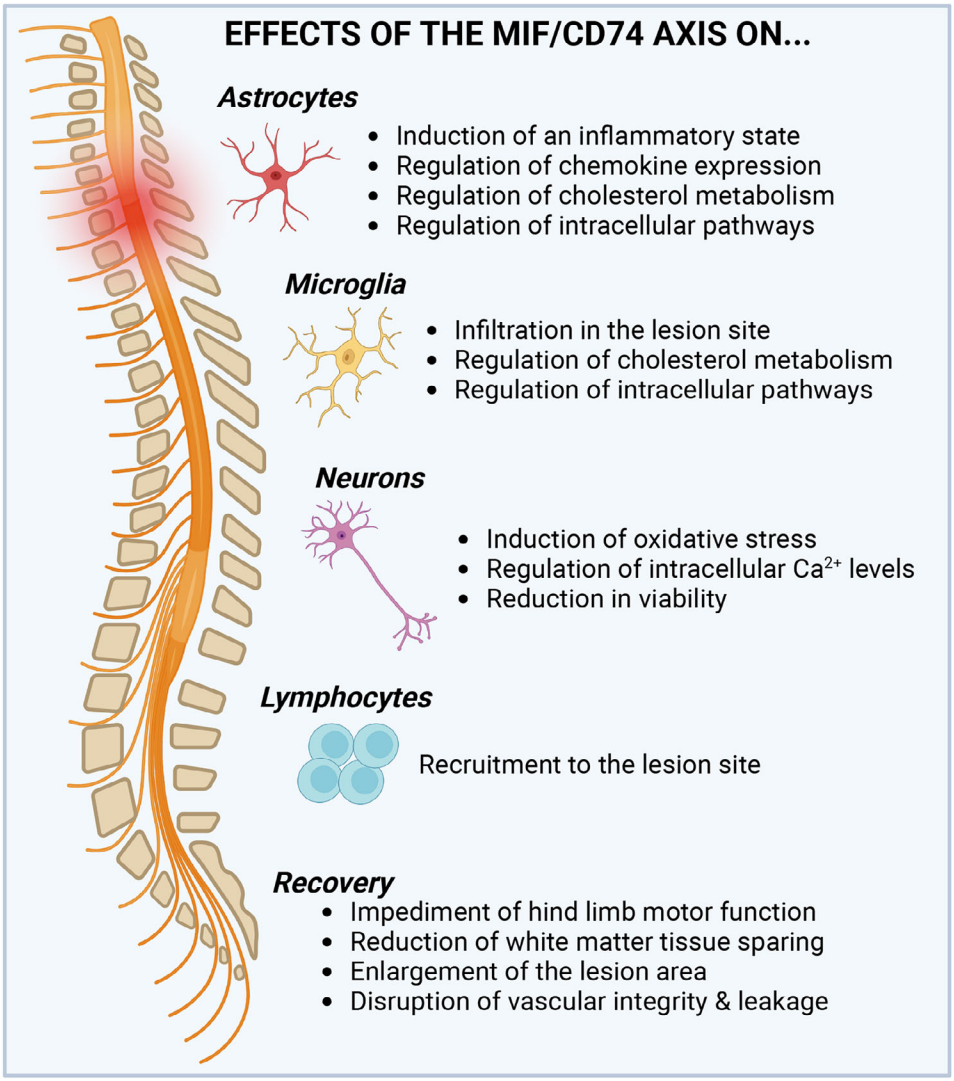

Figure 2. Overview of the effects of the MIF/CD74 axis on different cell types and recovery following traumatic SCI. The macrophage migration inhibitory factor (MIF)/CD74 axis affects astrocytes by inducing an inflammatory state and regulating chemokine expression, cholesterol metabolism, and intracellular pathways. Infiltration of microglia in the lesion site is influenced by MIF and CD74, as well as the regulation of microglial cholesterol metabolism and intracellular pathways. In neurons, MIF and CD74 induce oxidative stress, regulate intracellular Ca^{2+} levels, and reduce neuronal viability. The MIF/CD74 axis is also involved in the recruitment of lymphocytes to the lesion site. Lastly, recovery following traumatic SCI is affected by the axis through the impediment of hind limb motor function, reduction of white matter tissue sparing, enlargement of the lesion area, and disruption of vascular integrity and leakage.

and transection SCI models are popular. Contusion SCI models induce transient, acute injury to the spinal cord, whereas compression SCI models are characterized by sustained compression of the spinal cord over a longer period of time. For transection SCI models, the spinal cord is either completely or partially cut at a specific level [53]. In these SCI models, MIF/CD74 axis activation has been reported to have effects on astrocytes, microglia, neurons, lymphocytes, and even functional recovery, as discussed below (Fig. 2).

The MIF/CD74 axis in traumatic SCI rat models

The following section provides an overview of studies focusing on the MIF/CD74 axis in traumatic SCI rat models (Supporting Information Table S1). Changes in MIF and CD74 expression after experimental SCI, as well as their direct and indirect involvement in astrocyte and microglia function, are reviewed. Furthermore, the effects of MIF inhibition on functional improvement and pain regulation are discussed. Next to genetic manipulation of MIF expression, several small molecule inhibitors of MIF have been used in literature. Both 4-iodo-6-phenylpyrimidine (4-IPP), an irreversible antagonist, and ISO-1, a competitive antagonist, inhibit MIF tautomerase activity by binding to different regions of the hydrophobic binding pocket of the MIF trimer [54–56]. Direct effects of 4-IPP and ISO-1 on MIF binding to CD74 have not been studied to date, although the tautomerase active site of MIF has been shown to be important for receptor binding and signal transduction [57]. In addition, the allosteric inhibitor Chicago Sky Blue (CSB) was shown to occupy the interface of two MIF trimers, blocking both MIF's tautomerase activity and its binding to CD74 [54], [58].

MIF and CD74 expression after experimental SCI

In 2004, the first study focused on MIF in Wistar rats with a T8 compression SCI [24]. Spinal cord MIF mRNA expression,

as determined by Northern blot, was significantly elevated at 3 days postinjury compared with both 6 h postinjury and uninjured spinal cord tissue. In situ hybridization and/or immunohistochemistry (IHC) showed a peak in MIF expression at 3 days post-SCI in microglia that accumulated in the lesion epicenter and in astrocytes located in the preserved white matter surrounding the lesion. One week postinjury, some of the observed microglia and astrocytes in or around the cystic cavity were also positive for MIF [24]. More recently, MIF protein expression after SCI has been described in several research papers focusing on the role of MIF in astrocyte function post-SCI [59–65]. Here, Sprague Dawley rats were subjected to a T9 contusion SCI, and MIF protein expression in injured spinal cord tissue was measured with western blot or ELISA at 0, 1, 4, and 7 days postinjury. In all seven studies, a peak in MIF expression was detected at 4 days postinjury. MIF expression decreased thereafter at 7 days postinjury but was still significantly elevated compared with 0 days postinjury in 6 out of 7 studies [59], [61]–[65]. Similarly, MIF expression was already significantly increased at 1 day versus 0 days post-SCI in 5 out of 7 studies [60–64]. Immunostaining revealed that MIF colocalized with microglia; however, no information on the specific timepoints of the analysis was given [59]. Here, overlapping signals for MIF and astrocytes were found at 0, 1, 4, and 7 days after SCI [59], [63]. Under hypoxic conditions, MIF RNA and protein expression were significantly increased in primary spinal cord astrocytes isolated from Sprague Dawley rats [65]. Furthermore, a microarray dataset of thoracic propriospinal neurons isolated at 3 days, 2 weeks, and 1 month postinjury from Long Evans rats with a low-thoracic transection SCI revealed upregulation of CD74 gene expression at 3 days postinjury [66]. Lastly, in silico analysis of single-cell transcriptome data showed that MIF signaling is important for communication between cells (e.g., microglia and astrocytes) in spinal cord tissue of Sprague Dawley rats subjected to a T10 transection SCI [67]. It can be concluded that MIF and CD74 expression are upregulated shortly after SCI in rats, both in astrocytes and microglia. However, the type of SCI (i.e. compression, contusion, or transection) might affect the exact timing and cellular expression of MIF and CD74. In addition, MIF and CD74 signaling might also play a role in other cell types involved in traumatic SCI, which has not been investigated thus far.

Direct effects of the MIF/CD74 axis on astrocyte and microglia function

First, the functional involvement of MIF in relation to astrocyte function has been studied in rats [59]. Stimulation of primary rat spinal cord astrocytes with recombinant MIF (rMIF) increased the expression levels of both TNF-α and IL-1β mRNA and protein in a concentration-dependent manner. Elevated NF-κB protein expression following rMIF stimulation indicated a potential role for NF-κB signaling in mediating the effects of MIF on astrocytes. Co-immunoprecipitation experiments showed that MIF binds to the CD74 surface receptor on primary astrocytes, and knockdown of CD74 resulted in decreased TNF-α and IL-1β mRNA expression, NF-κB protein expression, and ERK activation. rMIF stimulation also led to increased proliferation of primary astrocytes [59]. Moreover, injection of 4-IPP at the lesion site of Sprague Dawley rats with a T9 contusion SCI led to a significant decrease in CC-motif chemokine ligand 5 (CCL5) and CCL2 in astrocytes inside the lesion [60], [64]. This finding was confirmed in primary astrocytes, in which transcriptome analysis indicated that both chemokines were regulated by the MIF/CD74 axis through the c-Jun N-terminal kinase (JNK) pathway, which is also a MAPK signaling pathway [60], [64]. Besides its effect on the inflammatory state of astrocytes, MIF was also shown to have an effect on microglia migration. Using an in vitro transwell migration assay, migration of RAW 264.7 macrophages and BV2 microglia was significantly increased in the presence of rMIF [60], [64]. In vivo, injection of MIF or CCL5 into the lesion site resulted in increased levels of microglia in the injured spinal cord, which was significantly reduced by 4-IPP injection [60], [64]. These results suggest that MIF regulates inflammatory cytokine and chemokine production in spinal cord astrocytes, and migration of microglia toward the injury site following SCI.

Indirect effects of the MIF/CD74 axis on astrocyte and microglia function

MIF has been shown to have an effect on cholesterol homeostasis, which is mainly regulated by astrocytes in the CNS [68]. It is hypothesized that, because of damage to the CNS, excessive cholesterol is released and converted by cholesterol-25-hydroxylase (CH25H) to 25-hydroxy-cholesterol (25-HC) [61]. Interestingly, 25-HC is involved in inflammatory responses, such as foam cell formation and inflammasome activation [69], [70]. MIF stimulation of primary spinal cord astrocytes increased 25-HC production via interaction with the CD74 receptor, which consequently resulted in increased production of the chemokine CCL5 [61]. Indeed, 25-HC stimulated the migration capacity of astrocytes in vitro but inhibited cell viability in a concentration-dependent manner. Moreover, two separate studies reported reduced CH25H gene and mRNA expression in MIF-stimulated primary spinal cord astrocytes with a CD74 siRNA knockdown [59], [61]. This indicates that the production of 25-HC is increased in response to MIF stimulation via the regulation of CH25H [61]. In an in vivo study, CH25H mRNA expression increased in the injured spinal cord from 1 to 7 days postinjury in astrocytes, whereas local 4-IPP injection significantly decreased CH25H expression at all time points [61]. These data show that the MIF/CD74 axis can potentially aggravate neuropathology by tightly regulating cholesterol homeostasis and, more specifically, CH25H and 25-HC expression, which will result in astrocyte migration.

MIF and CD74 can also affect the cyclooxygenase 2 (COX2)/prostaglandin E2 (PGE_2) pathway. COX2 is an enzyme that is expressed in the normal CNS but is highly induced by proinflammatory stimuli [62]. Together with the microsomal PGE synthase-1 (mPGES-1) enzyme, COX2 catalyzes the conversion of

arachidonic acid to PGE_2 [62]. PGE_2 has proinflammatory functions, as it can promote local vasodilation, inflammatory edema, and leukocyte activation, as well as anti-inflammatory functions, for example, by inducing IL-10 and inhibiting TNF-α production in macrophages [71], [72]. MIF-stimulated primary spinal cord microglia showed dose- and time-dependent increases in COX2 and mPGES-1 expression, and PGE_2 production, which were counteracted by COX2 inhibition via NS398 or siRNA-mediated knockdown of CD74 [73]. In addition, inhibition of MIF with ISO-1 also significantly reduced COX2 protein expression [73]. Regulation of the COX2/PGE_2 pathway by MIF/CD74 occurs via the MAPK pathways, more specifically the ERK and P38 pathways, as both ERK and P38 inhibition of MIF-stimulated primary microglia resulted in decreased COX2 and mPGES-1 expression, and PGE_2 production [73]. In an in vivo model of Sprague Dawley rats with a T9 contusion SCI, COX2 and mPGES-1 protein expression, as well as PGE_2 levels, significantly increased, peaking at 4 days post-SCI, whereas 4-IPP injection at the lesion site led to a significant decrease of all proteins at all measured time points (0, 1, 4, and 7 days postinjury) [62]. Here, COX2 expression colocalized with astrocytes in the lesion [62]. Furthermore, 4-IPP, NS398, CD74 siRNA knockdown, and MAPK pathway inhibitors attenuated the MIF-induced expression of COX2, mPGES-1, and PGE_2 in primary astrocytes [62]. Additionally, injection of NS398 at the lesion site significantly decreased PGE_2 and IL-1β, and increased TNF-α production in spinal cord tissue at 1, 4, and 7 days postinjury [62]. Further characterization of functional recovery and overall inflammation is needed to analyze the impact of COX-2 inhibition and the net effect of these contradictory cytokine changes on the disease course. Still, these results confirm that MIF can indirectly affect the inflammatory responses of other immune cells by regulating the COX2/PGE_2 axis via MAPK pathways in astrocytes and microglia.

Effects of the MIF/CD74 axis on functional improvement and pain regulation

The potential value of MIF inhibition as a therapeutic intervention has been indicated. In Sprague Dawley rats subjected to a T9 contusion SCI, injection of 4-IPP resulted in a significant improvement of hind limb motor function, as well as the occurrence of significantly smaller cystic cavities in the injured spinal cord, as opposed to rMIF or rCCL5 injection [60], [64]. Another study used CSB encapsulated in nanosized liposomes (nano-CSB) for intravenous delivery 48 h postinjury to Sprague Dawley rats with a T9 contusion SCI [74]. Nano-CSB-treated rats showed better preservation of vascular integrity with less leakage at the epicenter and at sites away from the injury, improved white matter tissue sparing, and thus a smaller wound area [74]. Although different outcome parameters were examined in these studies, the smaller cystic cavity after 4-IPP injection and improved white matter tissue sparing following nano-CSB treatment both indicate that MIF inhibition can reduce the secondary tissue damage following SCI. Next, MIF has been proposed to play a complex role in pain regulation after SCI. Low MIF levels were shown to induce hyperexcitability in primary sensory neurons, derived from the dorsal root ganglia of Sprague Dawley rats, while high levels triggered a hypoexcitable state [75]. ISO-1 treatment of these neurons blocked the SCI-induced hyperexcitability after a T10 contusion injury. Moreover, conditioned place aversion (i.e. learned aversion to a place associated with MIF injection) suggested that MIF stimulates affective pain [75]. Overall, these data suggest that interference of MIF signaling potentially has beneficial effects on motor function and tissue integrity, and might regulate pain after traumatic SCI. Additional large-scale studies with comparable injury type, injury level, animal strain, MIF blocking mechanism, and outcome parameters are needed to more clearly assess the therapeutic effect of MIF inhibition on traumatic SCI.

The MIF/CD74 axis in traumatic SCI mouse models

The following section provides an overview of studies focusing on the MIF/CD74 axis in traumatic SCI mouse models (Supporting Information Table S2). We discuss the effects of genetic manipulation of MIF and CD74 expression on neuronal viability, inflammation, and functional recovery.

Effects of MIF gene manipulation on neuronal viability and functional recovery

In primary mouse neurons, stimulation with rMIF resulted in a dose-dependent increase in cellular oxidation, intracellular calcium levels, and apoptosis and a decrease in neuronal viability [22]. Neuronal cell death was also measured in the spinal cord tissue of MIF KO and WT BALB/c mice with a T7-8 compression SCI [25]. IHC analysis using caspase-3 revealed significantly fewer apoptotic neuronal cells in the lesion of MIF KO mice than in those of WT mice at both 24 and 72 h postinjury. Additionally, cresyl-violet staining indicated better survival, although not significant, of motor neurons (i.e. diameter $\geq$ 30 μm) in the spinal cord lesion of MIF KO mice at 42 days postinjury [25]. This study also showed significantly improved hind limb locomotor recovery of MIF KO mice from 3 to 6 weeks post-SCI [25]. Although CD74 is expressed at low levels in human neurons and CD74 gene expression was shown to be upregulated in neurons from transection SCI rats, as discussed in Section "MIF and CD74 expression after experimental SCI", it is not clear yet whether the effects of MIF on neuronal viability are directly mediated by CD74 binding [66], [76], [77]. Further, sustainable release of MIF siRNA at the lesion site using injectable lipid nanoparticle hydrogel scaffolds in BALB/c mice with a T10 transection SCI significantly improved functional recovery up to 15 days postinjury compared with non-treated SCI mice [78]. Additionally, partial tissue regeneration in the spinal cord lesion area was observed, and IHC analysis of the injured spinal cord tissue revealed fewer microglia and astrocytes, and more (surviving) neurons in the rostral, lesion, and caudal sections of MIF siRNA-treated SCI mice. Despite the inclusion of 10 animals per group, only one image was shown for each group,

and no image analyses were performed [78]. Furthermore, in silico analysis of single-cell transcriptome data revealed a role for MIF and CD74 in microglia-astrocyte communication during glial scar formation in C57BL/6J mice with a T10 right lateral hemisection SCI [79].

Traumatic SCI often leads to increased oxidative stress, which can cause DNA damage [80]. Subsequently, DNA damage repair mechanisms are initiated, resulting in hyperactivation of poly(ADP-ribose) polymerase-1 (PARP-1) [36]. In turn, PARP-1 induces the mitochondrial release of the apoptosis-inducing factor (AIF), which forms a complex with cytosolic MIF. This complex translocates to the nucleus, where MIF, through its nuclease activity, induces DNA fragmentation and parthanatos, a form of regulated cell death [36]. Several in vitro studies using DNA damage inducers and PARP-1 inhibitors on spinal cord neurons and tissue have already implicated parthanatos in post-SCI pathology [81–83]. More recently, mouse neuro-2A neuroblastoma cells with a short hairpin (sh)RNA-mediated AIF or MIF knockdown were treated with H_2O_2, an inducer of parthanatos [80]. H_2O_2 treatment increased AIF and MIF nuclear translocation and parthanatos-mediated cell death, which were both reduced in their respective knockdown cell groups. Additionally, MIF knockdown significantly reduced single- and double-stranded DNA breaks in H_2O_2-treated neuroblastoma cells [80], which might explain the increase in neuronal cell death following MIF stimulation reported in prior publications, as mentioned above.

Effects of CD74 gene manipulation on inflammation and functional recovery

Mice are the preferred species for genetic studies, although choosing the right mouse strain is highly important as genetic manipulation of CD74 in different strains resulted in opposite outcomes after contusion SCI [84]. Here, C57BL/6J mice, with a class II MHC I-A^b genetic background, and BALB/c mice, with a class II MHC I-A^d genetic background, were modified to express only low levels of CD74 [84]. After a T12 contusion SCI, C57BL/6J mice recovered better than their matched WT controls, while BALB/c mice showed worse recovery than control mice. Moreover, a similar inverse effect was observed for B- and T cell recruitment kinetics (earlier in C57BL/6J mice) into the injured spinal cord and the infiltration of microglia at the lesion site (higher in C57BL/6J mice) [84].

The MIF/CD74 axis in traumatic SCI human studies

Several groups have determined MIF plasma or serum levels at different stages of SCI in patients. The first study determined MIF levels in the plasma of 18 SCI patients and 18 uninjured controls [85]. Longitudinal daily plasma samples were available for the SCI patients up to 15 days postinjury. Plasma MIF levels were significantly elevated up to 11 days postinjury in acute SCI patients compared with those in uninjured controls [85]. In a second study, significantly higher MIF levels were detected in the plasma of 207 acute SCI patients (median time since injury was 16 h) compared with 100 healthy controls [86]. MIF levels were also positively correlated with C-reactive protein and IL-6 levels as well as injury severity (measured by AIS grade). MIF was further proposed as a predictive marker (area under the receiver operating characteristic (ROC) curve (AUC): 0.73) for deep vein thrombosis (DVT), a common fatal complication of SCI [86]. No significant difference in MIF serum levels was found between subacute SCI patients ($n = 28$, 2 weeks postinjury) and healthy controls ($n = 16$) [87]. This finding is consistent with the results of the first study that reported a lack of significant differences in MIF plasma levels between SCI patients and uninjured individuals from 11 to 15 days postinjury [85]. However, the highest MIF serum levels were measured in AIS grade B patients compared with healthy controls, which conflicts with the data reported for acute SCI patients that showed higher MIF levels in AIS grade A patients than in AIS grade B, C, and D SCI patients [86], [87]. This might be due to differences in the timing of the samples, cohort sizes (acute SCI study: $n = 207$ patients versus subacute SCI study: $n = 28$ patients), average age, inclusion of all AIS grades (acute SCI study) versus only AIS grades A and B (subacute SCI study), use of plasma (acute SCI study) versus serum (subacute SCI study) and assays (acute SCI study: ELISA versus subacute SCI study: Luminex assay) [86], [87]. MIF plasma levels were also determined in 22 chronic SCI patients (>1 year postinjury with a mean of 12 years postinjury) and 19 uninjured individuals using a Luminex assay [88]. Higher median levels of MIF were observed in chronic SCI patients [88]. Recently, we also determined MIF levels in the plasma of 18 traumatic SCI patients at 0–4 days, 3, 6, 12, 18, and >18 weeks postinjury, and in 18 healthy controls by ELISA [89]. No significant differences were found between SCI patients in the (sub)acute (0–4 days to 3 weeks post-SCI) and chronic (6 to >18 weeks post-SCI) stage, and healthy individuals [89]. This lack of increased MIF levels is probably due to the low number of included SCI patients. An overview of the human studies that investigated the MIF/CD74 axis in traumatic SCI is shown in Supporting Information Table S3.

In conclusion, MIF levels were elevated in SCI patients compared with those in uninjured individuals [85], [86], [88]. However, not all studies found significant differences in MIF levels [87], [89]. Contradictory results have also been reported for the correlation of MIF and injury severity, as measured by the AIS [86], [87]. These differences might be due to the variation in patient characteristics or numbers between the different studies but might also depend on the type of assay or sample that was used. It can also be suggested that MIF expression rapidly but transiently increases after SCI and is re-expressed during the chronic stage. MIF levels may also strongly fluctuate throughout the SCI stages in patients with more severe injuries and remain more stable in patients with less severe injuries. Finally, MIF levels were associated with the development of DVT [86].

The MIF/CD74 axis in B cells

Over the years, MIF and CD74 have been extensively studied in B cells in both health and disease. Hereby, the effect of MIF/CD74 signaling on B cell function has been identified through stimulatory, blocking, silencing, and genetic studies. Under normal conditions, human primary B cells showed significantly reduced CD74 surface expression when treated with the MIF inhibitor ISO-1. Conversely, blocking CD74 with an anti-CD74 antibody resulted in decreased MIF mRNA expression [46]. With respect to intracellular signaling, MIF stimulation led to increased ERK1/2 and ZAP-70 phosphorylation in Raji B cells (a Burkitt lymphoma-derived lymphoblast cell line), and increased Akt and ZAP-70 phosphorylation in WT murine splenic B cells, which was inhibited by interfering with CD74, CD44 and CXCR4 signaling using either blocking antibodies or MIF receptor-specific KO mice [23], [33], [40]. MIF also induced complex formation between CD74-ICD and the transcription factor Paired Box 5 (PAX-5) in WT murine splenic B cells, which can then translocate to the cell nucleus and bind to the promoter region of the tumor suppressor gene Cyclin-D-binding Myb-like transcription factor 1 (DMTF1) [90]. In splenic B cells of CD74 KO mice or after PAX-5 siRNA knockdown, the MIF-induced downregulation of DMTF1 mRNA was reversed [90]. Furthermore, MIF and CD74 are highly involved in B cell survival. RNA expression of the anti-apoptotic genes Bcl-X_L and Bcl-2 was upregulated in WT splenic B cells after rMIF stimulation but was absent in splenic B cells treated with ISO-1, anti-CD74 or anti-CD44 blocking antibodies or those derived from CD74 or CD44 KO mice [40], [41]. Similar results were found for Bcl-2 mRNA expression in primary B cells of chronic lymphocytic leukemia (CLL) patients after rMIF stimulation, and after ISO-1 or anti-CD74 blocking [91]. In addition, MIF and CD74 were shown to control Bcl-2 expression through the regulation of IL-8 production in CLL-derived B cells [91]. Interference with MIF (ISO-1), CD74 (CD74 KO mice, stimulatory or blocking antibodies), CD44 (CD44 KO mice), or CXCR4 (blocking antibody) signaling in B cells revealed that this axis is required to ensure B cell survival, as shown by cell apoptosis analysis with propidium iodide and Annexin V [40], [91], [92]. Besides its involvement in survival, the MIF/CD74 axis also regulates B cell proliferation. rMIF stimulation induced the proliferation of Raji cells and splenic B cells, which was counteracted by ISO-1 and an anti-CD74 blocking antibody, while reduced splenic B cell proliferation was reported in CD74 and CD44 KO mice [33], [40]. rMIF-induced S-phase cell cycle entry and upregulation of cyclin-E RNA expression were also inhibited in ISO-1-treated splenic B cells or those derived from CD74 or CD44 KO mice [40]. Next, in vitro transwell assays showed decreased MIF-guided chemotaxis of B cells after interference with MIF, CD74, CXCR4, or ZAP-70 signaling [23], [92]. In murine splenic B cells, MIF further induced intracellular calcium release and F-actin polymerization, which are needed for migration [23]. Lastly, in silico analysis of available single-cell transcriptome data showed that the MIF/CD74 axis is highly involved in the communication between B cells and tumor cells or cells present in the aortic aneurysm microenvironment [93], [94].

However, the role of the MIF/CD74 axis in B cells in traumatic SCI remains undetermined. Recently, we reported significantly increased frequencies of $CD74^+$ cells, as well as significantly increased CD74 surface expression, within circulating total $CD19^+$ B cells and B cell subsets in both (sub)acute ($\leq$1 month postinjury) and chronic (>1 month postinjury) SCI patients using high-dimensional flow cytometry [89]. This finding highlights the potential role of the MIF/CD74 axis in post-SCI B cell responses. B cells are involved in the secondary inflammatory responses that are induced following an SCI, as indicated by their presence in spinal cord tissue at both the acute and chronic stages after traumatic SCI, the attenuated functional deficits, and reduced pathology that are evident in SCI mice depleted of B cells [95–98]. Thus, although the importance of the MIF/CD74 axis in B cell functioning has been elaborately demonstrated, research in the context of SCI is missing. Based on the known effects of MIF/CD74 signaling on B cell function and the upregulation of both MIF (in the spinal cord) and CD74 (on circulating B cells) expression following traumatic SCI, we propose the following working model. Following a traumatic SCI, MIF is produced locally inside the injured spinal cord, mainly by astrocytes and microglia. Because of disturbances of the blood-spinal cord barrier post-SCI, MIF, together with CNS proteins, can also be released into the circulation. In the peripheral lymphoid tissues, these CNS proteins can (re)activate autoreactive B cells, after which MIF could stimulate the proliferation and survival of these cells by binding to the CD74 surface receptor. MIF could then direct these autoreactive B cells toward the spinal cord lesion site, where they can contribute to the secondary injury cascade post-SCI. Research into the exact role of MIF/CD74 signaling in post-SCI B cell responses using both human and animal studies is needed to clarify the potential importance of this axis following SCI and could lead to novel insights into SCI pathology and potentially also to novel therapeutic targets.

Conclusion

Overall, the role of MIF in traumatic SCI has been highlighted in both animal and human studies. By acting through CD74, MIF can promote pro- and anti-inflammatory outcomes, directly or indirectly, via the activation of numerous downstream pathways in different cell types. MIF has been found in spinal cord tissue and cells residing in or near the lesion site, and inhibition or knockout of this cytokine improved functional recovery and tissue integrity after traumatic SCI. However, careful choices must be made when performing animal studies, as experimental outcomes can be affected by the type of injury and the choice of animal strain. In human studies, MIF levels have been found to be elevated in SCI patients and were correlated with the development of DVT. However, there are still gaps in our knowledge about the MIF/CD74 axis in traumatic SCI. Efforts to find clinically relevant correlations have already been made, but further investigation of the MIF/CD74 axis on a larger scale is required for potential clinical implementation. Research on MIF in SCI has focused on astrocytes, microglia, and neurons. Therefore, broadening toward

other cell types, such as B cells, is highly needed, as research already showed an important role for the MIF/CD74 axis in B cell functioning. More recently, we revealed increased CD74 expression on B cells in traumatic SCI, which provides a new path for investigation. This shift might lead to a better understanding of how MIF and CD74 contribute to the secondary injury cascade following traumatic SCI. Specialized therapies targeting MIF (imalumab) and/or CD74 (milatuzumab) are already being tested in clinical trials for other diseases, mainly cancers, and might also benefit thousands of traumatic SCI patients worldwide [99], [100].

Acknowledgements: This work was supported by Hasselt University, Belgium, and by a grant from the Wings for Life Spinal Cord Research Foundation (WFL-BE-02/22). Figures were created with BioRender.com.

Conflict of interest: The authors declare no conflict of interest.

Author contributions: Serina Rubio and Judith Fraussen wrote the manuscript. Judith Fraussen and Veerle Somers supervised. Serina Rubio, Judith Fraussen, and Veerle Somers reviewed and edited the manuscript. All authors read and approved the final manuscript.

Permission to reproduce material from other sources: The data and materials used in this review article were obtained from published articles found on PubMed.

Data availability statement: Data sharing is not applicable to this review article as no new data were created or analyzed in this study.

References

1 WHO. Spinal Cord Injury. [updated 19 November 2013; cited 2022. Available from: https://www.who.int/news-room/fact-sheets/detail/spinal-cord-injury

2 National Spinal Cord Injury Statistical Center. *Traumatic Spinal Cord Injury - Facts and Figures at a Glance*. University of Alabama at Birmingham. 2023.

3 **Dukes, E. M., Kirshblum, S., Aimetti, A. A., Qin, S. S., Bornheimer, R. K. and Oster, G.**, Relationship of American Spinal Injury Association Impairment Scale Grade to post-injury hospitalization and costs in thoracic spinal cord injury. *Neurosurgery*. 2018. **83**: 445–451.

4 National Institute of Neurological Disorders and Stroke. Spinal Cord Injury: Hope Through Research. 2013 [updated 2021-06-04. Available from: https://www.ninds.nih.gov/Disorders/Patient-Caregiver-Education/Hope-Through-Research/Spinal-Cord-Injury-Hope-Through-Research

5 National Institute of Child Health and Human Development. What are the treatments for spinal cord injury (SCI)? [updated 12/1/2016; cited 2022. Available from: https://www.nichd.nih.gov/health/topics/spinalinjury/conditioninfo/treatments

6 **Roberts, T. T., Leonard, G. R. and Cepela, D. J.**, Classifications in brief: American Spinal Injury Association (ASIA) Impairment Scale. *Clin. Orthop. Relat. Res*. 2017. **475**: 1499–1504.

7 **Ahuja, C. S., Wilson, J. R., Nori, S., Kotter, M. R. N., Druschel, C., Curt, A., Fehlings, M. G. et al.**, Traumatic spinal cord injury. *Nat. Rev. Dis. Primers*. 2017;**3**: 17018.

8 **Quadri, S. A., Farooqui, M., Ikram, A., Zafar, A., Khan, M. A., Suriya, S. S., Claus, C. F. et al.**, Recent update on basic mechanisms of spinal cord injury. *Neurosurg. Rev*. 2020. **43**: 425–441.

9 **Dusart, I. and Schwab, M. E.**, Secondary cell death and the inflammatory reaction after dorsal hemisection of the rat spinal cord. *Eur. J. Neurosci*. 1994. **6**: 712–724.

10 **Wolman, L.**, The disturbance of circulation in traumatic paraplegia in acute and late stages: a pathological study. *Paraplegia.* 1965;**2**: 213–226.

11 **Dohrmann, G. J., Wagner F. C., Jr and Bucy, P. C.**, The microvasculature in transitory traumatic paraplegia. An electron microscopic study in the monkey. *J. Neurosurg*. 1971. **35**: 263–271.

12 **Carlson, S. L., Parrish, M. E., Springer, J. E., Doty, K. and Dossett, L.**, Acute inflammatory response in spinal cord following impact injury. *Exp. Neurol*. 1998. **151**: 77–88.

13 **Popovich, P. G., Wei, P. and Stokes, B. T.**, Cellular inflammatory response after spinal cord injury in Sprague-Dawley and Lewis rats. *J Comp Neurol*. 1997. **377**: 443–464.

14 **Pineau, I. and Lacroix, S.**, Proinflammatory cytokine synthesis in the injured mouse spinal cord: multiphasic expression pattern and identification of the cell types involved. *J. Comp. Neurol*. 2007. **500**: 267–285.

15 **Faden, A. I. and Simon, R. P.**, A potential role for excitotoxins in the pathophysiology of spinal cord injury. *Ann. Neurol*. 1988. **23**: 623–626.

16 **Panter, S. S., Yum, S. W. and Faden, A. I.**, Alteration in extracellular amino acids after traumatic spinal cord injury. *Ann. Neurol*. 1990. **27**: 96–99.

17 **Tymianski, M., Charlton, M. P., Carlen, P. L. and Tator, C. H.**, Secondary Ca2+ overload indicates early neuronal injury which precedes staining with viability indicators. *Brain Res*. 1993;**607**: 319–323.

18 **Li, S., Mealing, G. A., Morley, P. and Stys, P. K.**, Novel injury mechanism in anoxia and trauma of spinal cord white matter: glutamate release via reverse Na+-dependent glutamate transport. *J. Neurosci*. 1999;**19**: RC16.

19 **Li, S. and Stys, P. K.**, Mechanisms of ionotropic glutamate receptor-mediated excitotoxicity in isolated spinal cord white matter. *J. Neurosci*. 2000. **20**: 1190–1198.

20 **Su, Z., Yuan, Y., Chen, J., Zhu, Y., Qiu, Y., Zhu, F., Huang, A. et al.**, Reactive astrocytes inhibit the survival and differentiation of oligodendrocyte precursor cells by secreted TNF-alpha. *J. Neurotrauma*. 2011. **28**: 1089–1100.

21 **Fawcett, J. W. and Asher, R. A.**, The glial scar and central nervous system repair. *Brain Res. Bull*. 1999. **49**: 377–391.

22 **Chalimoniuk, M., King-Pospisil, K., Metz, C. N. and Toborek, M.**, Macrophage migration inhibitory factor induces cell death and decreases neuronal nitric oxide expression in spinal cord neurons. *Neuroscience*. 2006. **139**: 1117–1128.

23 **Klasen, C., Ohl, K., Sternkopf, M., Shachar, I., Schmitz, C., Heussen, N., Hobeika, E. et al.**, MIF promotes B cell chemotaxis through the receptors CXCR4 and CD74 and ZAP-70 signaling. *J. Immunol*. 2014. **192**: 5273–5284.

24 **Koda, M., Nishio, Y., Hashimoto, M., Kamada, T., Koshizuka, S., Yoshinaga, K., Onodera, S. et al.**, Up-regulation of macrophage migration-inhibitory factor expression after compression-induced spinal cord injury in rats. *Acta Neuropathol*. 2004. **108**: 31–36.

25 **Nishio, Y., Koda, M., Hashimoto, M., Kamada, T., Koshizuka, S., Yoshinaga, K., Onodera, S. et al.**, Deletion of macrophage migration inhibitory factor attenuates neuronal death and promotes functional recovery after compression-induced spinal cord injury in mice. *Acta Neuropathol*. 2009. **117**: 321–328.

26 **Ogata, A., Nishihira, J., Suzuki, T., Nagashima, K. and Tashiro, K.**, Identification of macrophage migration inhibitory factor mRNA expression in neural cells of the rat brain by in situ hybridization. *Neurosci. Lett*. 1998. **246**: 173–177.

27 **Bloom, B. R. and Bennett, B.**, Mechanism of a reaction in vitro associated with delayed-type hypersensitivity. *Science*. 1966. **153**: 80–82.

28 **Lue, H., Kleemann, R., Calandra, T., Roger, T. and Bernhagen, J.**, Macrophage migration inhibitory factor (MIF): mechanisms of action and role in disease. *Microbes Infect*. 2002. **4**: 449–460.

29 **Basile, M. S., Battaglia, G., Bruno, V., Mangano, K., Fagone, P., Petralia, M. C., Nicoletti, F. et al.**, The dichotomic role of macrophage migration inhibitory factor in neurodegeneration. *Int. J. Mol. Sci*. 2020;**21**.

30 **Kindt, N., Journe, F., Laurent, G. and Saussez, S.**, Involvement of macrophage migration inhibitory factor in cancer and novel therapeutic targets. *Oncol. Lett*. 2016. **12**: 2247–2253.

31 **Calandra, T. and Roger, T.**, Macrophage migration inhibitory factor: a regulator of innate immunity. *Nat. Rev. Immunol*. 2003. **3**: 791–800.

32 **Weiser, W. Y., Temple, P. A., Witek-Giannotti, J. S., Remold, H. G., Clark, S. C. and David, J. R.**, Molecular cloning of a cDNA encoding a human macrophage migration inhibitory factor. *Proc. Natl. Acad. Sci. USA*. 1989. **86**: 7522–7526.

33 **Leng, L., Metz, C. N., Fang, Y., Xu, J., Donnelly, S., Baugh, J., Delohery, T. et al.**, MIF signal transduction initiated by binding to CD74. *J. Exp. Med*. 2003. **197**: 1467–1476.

34 **Meza-Romero, R., Benedek, G., Leng, L., Bucala, R. and Vandenbark, A. A.**, Predicted structure of MIF/CD74 and RTL1000/CD74 complexes. *Metab. Brain Dis*. 2016. **31**: 249–255.

35 **Rosengren, E., Bucala, R., Aman, P., Jacobsson, L., Odh, G., Metz, C. N., Rorsman, H. et al.**, The immunoregulatory mediator macrophage migration inhibitory factor (MIF) catalyzes a tautomerization reaction. *Mol. Med*. 1996. **2**: 143–149.

36 **Wang, Y., An, R., Umanah, G. K., Park, H., Nambiar, K., Eacker, S. M., Kim, B. et al.**, A nuclease that mediates cell death induced by DNA damage and poly(ADP-ribose) polymerase-1. *Science*. 2016;**354**.

37 **Jankauskas, S. S., Wong, D. W. L., Bucala, R., Djudjaj, S. and Boor, P.**, Evolving complexity of MIF signaling. *Cell Signal*. 2019;**57**: 76–88.

38 **Bucala, R. and Shachar, I.**, The integral role of CD74 in antigen presentation, MIF signal transduction and B cell survival and homeostasis. *Mini Rev. Med. Chem*. 2014. **14**: 1132–1138.

39 **Shi, X., Leng, L., Wang, T., Wang, W., Du, X., Li, J., McDonald, C. et al.**, CD44 is the signaling component of the macrophage migration inhibitory factor-CD74 receptor complex. *Immunity*. 2006. **25**: 595–606.

40 **Gore, Y., Starlets, D., Maharshak, N., Becker-Herman, S., Kaneyuki, U., Leng, L., Bucala, R. et al.**, Macrophage migration inhibitory factor induces B cell survival by activation of a CD74-CD44 receptor complex. *J. Biol. Chem*. 2008. **283**: 2784–2792.

41 **Starlets, D., Gore, Y., Binsky, I., Haran, M., Harpaz, N., Shvidel, L., Becker-Herman, S. et al.**, Cell-surface CD74 initiates a signaling cascade leading to cell proliferation and survival. *Blood*. 2006. **107**: 4807–4816.

42 **Becker-Herman, S., Arie, G., Medvedovsky, H., Kerem, A. and Shachar, I.**, CD74 is a member of the regulated intramembrane proteolysis-processed protein family. *Mol. Biol. Cell*. 2005. **16**: 5061–5069.

43 **Bernhagen, J., Krohn, R., Lue, H., Gregory, J. L., Zernecke, A., Koenen, R. R., Dewor, M. et al.**, MIF is a noncognate ligand of CXC chemokine receptors in inflammatory and atherogenic cell recruitment. *Nat. Med*. 2007. **13**: 587–596.

44 **Alampour-Rajabi, S., El Bounkari, O., Rot, A., Muller-Newen, G., Bachelerie, F., Gawaz, M., Weber, C. et al.**, MIF interacts with CXCR7 to promote receptor internalization, ERK1/2 and ZAP-70 signaling and lymphocyte chemotaxis. *FASEB J*. 2015. **29**: 4497–4511.

45 **Schwartz, V., Lue, H., Kraemer, S., Korbiel, J., Krohn, R., Ohl, K., Bucala, R. et al.**, A functional heteromeric MIF receptor formed by CD74 and CXCR4. *FEBS Lett*. 2009. **583**: 2749–2757.

46 **Rijvers, L., Melief, M. J., van der Vuurst de Vries, R. M., Stephant, M., van Langelaar, J., Wierenga-Wolf, A. F., Hogervorst, J. M. et al.**, The macrophage migration inhibitory factor pathway in human B cells is tightly controlled and dysregulated in multiple sclerosis. *Eur. J. Immunol*. 2018. **48**: 1861–1871.

47 **Klasen, C., Ziehm, T., Huber, M., Asare, Y., Kapurniotu, A., Shachar, I., Bernhagen, J. et al.**, LPS-mediated cell surface expression of CD74 promotes the proliferation of B cells in response to MIF. *Cell Signal*. 2018;**46**: 32–42.

48 **Lue, H., Thiele, M., Franz, J., Dahl, E., Speckgens, S., Leng, L., Fingerle-Rowson, G. et al.**, Macrophage migration inhibitory factor (MIF) promotes cell survival by activation of the Akt pathway and role for CSN5/JAB1 in the control of autocrine MIF activity. *Oncogene*. 2007. **26**: 5046–5059.

49 **Farr, L., Ghosh, S., Jiang, N., Watanabe, K., Parlak, M., Bucala, R., Moonah, S. et al.**, CD74 signaling links inflammation to intestinal epithelial cell regeneration and promotes mucosal healing. *Cell Mol. Gastroenterol. Hepatol*. 2020. **10**: 101–112.

50 **Marsh, L. M., Cakarova, L., Kwapiszewska, G., von Wulffen, W., Herold, S., Seeger, W., Lohmeyer, J. et al.**, Surface expression of CD74 by type II alveolar epithelial cells: a potential mechanism for macrophage migration inhibitory factor-induced epithelial repair. *Am. J. Physiol. Lung Cell Mol. Physiol*. 2009;**296**: L442–L452.

51 **Nishio, Y., Nishihira, J., Ishibashi, T., Kato, H. and Minami, A.**, Role of macrophage migration inhibitory factor (MIF) in peripheral nerve regeneration: anti-MIF antibody induces delay of nerve regeneration and the apoptosis of Schwann cells. *Mol. Med*. 2002. **8**: 509–520.

52 **Song, H., Zhu, Z., Zhou, Y., Du, N., Song, T., Liang, H., Chen, X. et al.**, MIF/CD74 axis participates in inflammatory activation of Schwann cells following sciatic nerve injury. *J. Mol. Histol*. 2019. **50**: 355–367.

53 **Cheriyan, T., Ryan, D. J., Weinreb, J. H., Cheriyan, J., Paul, J. C., Lafage, V., Kirsch, T. et al.**, Spinal cord injury models: a review. *Spinal Cord*. 2014. **52**: 588–595.

54 **Sumaiya, K., Langford, D., Natarajaseenivasan, K. and Shanmughapriya, S.**, Macrophage migration inhibitory factor (MIF): a multifaceted cytokine regulated by genetic and physiological strategies. *Pharmacol. Ther*. 2022;**233**: 108024.

55 **Winner, M., Meier, J., Zierow, S., Rendon, B. E., Crichlow, G. V., Riggs, R., Bucala, R. et al.**, A novel, macrophage migration inhibitory factor suicide substrate inhibits motility and growth of lung cancer cells. *Cancer Res*. 2008. **68**: 7253–7257.

56 **Lubetsky, J. B., Dios, A., Han, J., Aljabari, B., Ruzsicska, B., Mitchell, R., Lolis, E. et al.**, The tautomerase active site of macrophage migration inhibitory factor is a potential target for discovery of novel anti-inflammatory agents. *J. Biol. Chem*. 2002. **277**: 24976–24982.

57 **Dios, A., Mitchell, R. A., Aljabari, B., Lubetsky, J., O'Connor, K., Liao, H., Senter, P. D. et al.**, Inhibition of MIF bioactivity by rational design of

pharmacological inhibitors of MIF tautomerase activity. *J. Med. Chem*. 2002. **45**: 2410–2416.

58 **Bai, F., Asojo, O. A., Cirillo, P., Ciustea, M., Ledizet, M., Aristoff, P. A., Leng, L. et al.**, A novel allosteric inhibitor of macrophage migration inhibitory factor (MIF). *J. Biol. Chem*. 2012. **287**: 30653–30663.

59 **Su, Y., Wang, Y., Zhou, Y., Zhu, Z., Zhang, Q., Zhang, X., Wang, W. et al.**, Macrophage migration inhibitory factor activates inflammatory responses of astrocytes through interaction with CD74 receptor. *Oncotarget*. 2017. **8**: 2719–2730.

60 **Zhou, Y., Guo, W., Zhu, Z., Hu, Y., Wang, Y., Zhang, X., Wang, W. et al.**, Macrophage migration inhibitory factor facilitates production of CCL5 in astrocytes following rat spinal cord injury. *J. Neuroinflammation*. 2018. **15**: 253.

61 **Zhu, Z., Hu, Y., Zhou, Y., Zhang, Y., Yu, L., Tao, L., Guo, A. et al.**, Macrophage migration inhibitory factor promotes chemotaxis of astrocytes through regulation of cholesterol 25-hydroxylase following rat spinal cord injury. *Neuroscience*. 2019;**408**: 349–360.

62 **Zhang, Y., Zhou, Y., Chen, S., Hu, Y., Zhu, Z., Wang, Y., Du, N. et al.**, Macrophage migration inhibitory factor facilitates prostaglandin E2 production of astrocytes to tune inflammatory milieu following spinal cord injury. *J. Neuroinflammation*. 2019. **16**: 85.

63 **Yang, T., Jiang, H., Luo, X., Hou, Y., Li, A., He, B., Zhang, X. et al.**, Thrombin acts as inducer of proinflammatory macrophage migration inhibitory factor in astrocytes following rat spinal cord injury. *J .Neuroinflammation*. 2022. **19**: 120.

64 **Zhang, H., Hu, Y. M., Wang, Y. J., Zhou, Y., Zhu, Z. J., Chen, M. H., Wang, Y. J. et al.**, Macrophage migration inhibitory factor facilitates astrocytic production of the CCL2 chemokine following spinal cord injury. *Neural Regen. Res*. 2023. **18**: 1802–1808.

65 **Hao, H., Hou, Y., Li, A., Niu, L., Li, S., He, B., Zhang, X. et al.**, HIF-1alpha promotes astrocytic production of macrophage migration inhibitory factor following spinal cord injury. *CNS Neurosci. Ther*. 2023.

66 **Jin, L., Wu, Z., Xu, W., Hu, X., Zhang, J., Xue, Z., Cheng, L. et al.**, Identifying gene expression profile of spinal cord injury in rat by bioinformatics strategy. *Mol. Biol. Rep*. 2014. **41**: 3169–3177.

67 **Ye, J., Wen, Z., Wu, T., Chen, L., Sheng, L., Wang, C., Teng, C. et al.**, Single-Cell Sequencing Reveals the Optimal Time Window for Anti-Inflammatory Treatment in Spinal Cord Injury. *Adv. Biol. (Weinh)*. 2023: e2300098.

68 **Ferris, H. A., Perry, R. J., Moreira, G. V., Shulman, G. I., Horton, J. D. and Kahn, C. R.**, Loss of astrocyte cholesterol synthesis disrupts neuronal function and alters whole-body metabolism. *Proc. Natl. Acad. Sci. USA*. 2017. **114**: 1189–1194.

69 **Gold, E. S., Ramsey, S. A., Sartain, M. J., Selinummi, J., Podolsky, I., Rodriguez, D. J., Moritz, R. L. et al.**, ATF3 protects against atherosclerosis by suppressing 25-hydroxycholesterol-induced lipid body formation. *J. Exp. Med*. 2012. **209**: 807–817.

70 **Jang, J., Park, S., Jin Hur, H., Cho, H. J., Hwang, I., Pyo Kang, Y., Im, I. et al.**, 25-hydroxycholesterol contributes to cerebral inflammation of X-linked adrenoleukodystrophy through activation of the NLRP3 inflammasome. *Nat. Commun*. 2016;**7**: 13129.

71 **Rodriguez, M., Domingo, E., Municio, C., Alvarez, Y., Hugo, E., Fernandez, N., Crespo, M. S. et al.**, Polarization of the innate immune response by prostaglandin E2: a puzzle of receptors and signals. *Mol. Pharmacol*. 2014. **85**: 187–197.

72 **Strassmann, G., Patil-Koota, V., Finkelman, F., Fong, M. and Kambayashi, T.**, Evidence for the involvement of interleukin 10 in the differential deactivation of murine peritoneal macrophages by prostaglandin E2. *J. Exp. Med*. 1994. **180**: 2365–2370.

73 **Wang, F., Wu, H., Xu, S., Guo, X., Yang, J. and Shen, X.**, Macrophage migration inhibitory factor activates cyclooxygenase 2-prostaglandin E2 in cultured spinal microglia. *Neurosci. Res*. 2011. **71**: 210–218.

74 **Saxena, T., Loomis, K. H., Pai, S. B., Karumbaiah, L., Gaupp, E., Patil, K., Patkar, R. et al.**, Nanocarrier-mediated inhibition of macrophage migration inhibitory factor attenuates secondary injury after spinal cord injury. *ACS Nano*. 2015. **9**: 1492–1505.

75 **Bavencoffe, A. G., Spence, E. A., Zhu, M. Y., Garza-Carbajal, A., Chu, K. E., Bloom, O. E., Dessauer, C. W. et al.**, Macrophage migration inhibitory factor (MIF) makes complex contributions to pain-related hyperactivity of nociceptors after spinal cord injury. *J. Neurosci*. 2022. **42**: 5463–5480.

76 **Bryan, K. J., Zhu, X., Harris, P. L., Perry, G., Castellani, R. J., Smith, M. A., Casadesus, G. et al.**, Expression of CD74 is increased in neurofibrillary tangles in Alzheimer's disease. *Mol. Neurodegener*. 2008. **3**: 13.

77 Human Protein Atlas. CD74 [cited 2024. Available from: https://www.proteinatlas.org/ENSG00000019582-CD74

78 **Gao, Y., Wang, K., Wu, S., Wu, J., Zhang, J., Li, J., Lei, S. et al.**, Injectable and photocurable gene scaffold facilitates efficient repair of spinal cord injury. *ACS Appl. Mater. Interfaces*. 2024. **16**: 4375–4394.

79 **Gong, L., Gu, Y., Han, X., Luan, C., Liu, C., Wang, X., Sun, Y. et al.**, Spatiotemporal dynamics of the molecular expression pattern and intercellular interactions in the glial scar response to spinal cord injury. *Neurosci. Bull*. 2023. **39**: 213–244.

80 **Yang, D., Shu, T., Zhao, H., Sun, Y., Xu, W. and Tu, G.**, Knockdown of macrophage migration inhibitory factor (MIF), a novel target to protect neurons from parthanatos induced by simulated post-spinal cord injury oxidative stress. *Biochem. Biophys. Res. Commun*. 2020. **523**: 719–725.

81 **Kuzhandaivel, A., Nistri, A. and Mladinic, M.**, Kainate-mediated excitotoxicity induces neuronal death in the rat spinal cord in vitro via a PARP-1 dependent cell death pathway (Parthanatos). *Cell Mol. Neurobiol*. 2010. **30**: 1001–1012.

82 **Mazzone, G. L. and Nistri, A.**, Effect of the PARP-1 inhibitor PJ 34 on excitotoxic damage evoked by kainate on rat spinal cord organotypic slices. *Cell Mol. Neurobiol*. 2011. **31**: 469–478.

83 **Nasrabady, S. E., Kuzhandaivel, A., Mladinic, M. and Nistri, A.**, Effects of 6(5H)-phenanthridinone, an inhibitor of poly(ADP-ribose)polymerase-1 activity (PARP-1), on locomotor networks of the rat isolated spinal cord. *Cell Mol. Neurobiol*. 2011. **31**: 503–508.

84 **Schori, H., Shechter, R., Shachar, I. and Schwartz, M.**, Genetic manipulation of CD74 in mouse strains of different backgrounds can result in opposite responses to central nervous system injury. *J. Immunol*. 2007. **178**: 163–171.

85 **Bank, M., Stein, A., Sison, C., Glazer, A., Jassal, N., McCarthy, D., Shatzer, M. M. et al.**, Elevated circulating levels of the pro-inflammatory cytokine macrophage migration inhibitory factor in individuals with acute spinal cord injury. *Arch. Phys. Med. Rehabil*. 2015. **96**: 633–644.

86 **Wu, D. M., Zheng, Z. H., Wang, S., Wen, X., Han, X. R., Wang, Y. J., Shen, M. et al.**, Association between plasma macrophage migration inhibitor factor and deep vein thrombosis in patients with spinal cord injuries. *Aging*. 2019. **11**: 2447–2456.

87 **Ogurcov, S., Shulman, I., Garanina, E., Sabirov, D., Baichurina, I., Kuznetcov, M., Masgutova, G. et al.**, Blood serum cytokines in patients with subacute spinal cord injury: a pilot study to search for biomarkers of injury severity. *Brain Sci*. 2021;**11**.

88 **Stein, A., Panjwani, A., Sison, C., Rosen, L., Chugh, R., Metz, C., Bank, M. et al.**, Pilot study: elevated circulating levels of the proinflammatory cytokine macrophage migration inhibitory factor in patients with chronic spinal cord injury. *Arch Phys. Med. Rehabil*. 2013. **94**: 1498–1507.

89 **Fraussen, J., Beckers, L., van Laake-Geelen, C. C. M., Depreitere, B., Deckers, J., Cornips, E. M. J., Peuskens, D. et al.**, Altered Circulating immune cell distribution in traumatic spinal cord injury patients in relation to clinical parameters. *Front. Immunol*. 2022;**13**: 873315.

90 **David, K., Friedlander, G., Pellegrino, B., Radomir, L., Lewinsky, H., Leng, L., Bucala, R. et al.**, CD74 as a regulator of transcription in normal B cells. *Cell Rep*. 2022. **41**: 111572.

91 **Binsky, I., Haran, M., Starlets, D., Gore, Y., Lantner, F., Harpaz, N., Leng, L. et al.**, IL-8 secreted in a macrophage migration-inhibitory factor- and CD74-dependent manner regulates B cell chronic lymphocytic leukemia survival. *Proc. Natl. Acad. Sci. USA.* 2007. **104**: 13408–13413.

92 **Thavayogarajah, T., Sinitski, D., El Bounkari, O., Torres-Garcia, L., Lewinsky, H., Harjung, A., Chen, H.-R. et al.**, CXCR4 and CD74 together enhance cell survival in response to macrophage migration-inhibitory factor in chronic lymphocytic leukemia. *Exp. Hematol*. 2022;**115**: 30–43.

93 **Bai, Y., Chen, D., Cheng, C., Li, Z., Chi, H., Zhang, Y., Zhang, X. et al.**, Immunosuppressive landscape in hepatocellular carcinoma revealed by single-cell sequencing. *Front. Immunol*. 2022;**13**: 950536.

94 **Cao, G., Lu, Z., Gu, R., Xuan, X., Zhang, R., Hu, J., Dong, H. et al.**, Deciphering the intercellular communication between immune cells and altered vascular smooth muscle cell phenotypes in aortic aneurysm from single-cell transcriptome data. *Front. Cardiovasc. Med*. 2022;**9**: 936287.

95 **Fisher, E. S., Amarante, M. A., Lowry, N., Lotz, S., Farjood, F., Temple, S., Hill, C. E. et al.**, Single cell profiling of CD45(+) spinal cord cells reveals microglial and B cell heterogeneity and crosstalk following spinal cord injury. *J. Neuroinflammation*. 2022. **19**: 266.

96 **Ankeny, D. P., Guan, Z. and Popovich, P. G.**, B cells produce pathogenic antibodies and impair recovery after spinal cord injury in mice. *J. Clin. Invest*. 2009. **119**: 2990–2999.

97 **Zrzavy, T., Schwaiger, C., Wimmer, I., Berger, T., Bauer, J., Butovsky, O., Schwab, J. M. et al.**, Acute and non-resolving inflammation associate with oxidative injury after human spinal cord injury. *Brain*. 2021. **144**: 144–161.

98 **Casili, G., Impellizzeri, D., Cordaro, M., Esposito, E. and Cuzzocrea, S.**, B-cell depletion with CD20 antibodies as new approach in the treatment of inflammatory and immunological events associated with spinal cord injury. *Neurotherapeutics*. 2016. **13**: 880–894.

99 **Mahalingam, D., Patel, M. R., Sachdev, J. C., Hart, L. L., Halama, N., Ramanathan, R. K., Sarantopoulos, J. et al.** Phase I study of imalumab (BAX69), a fully human recombinant antioxidized macrophage migration inhibitory factor antibody in advanced solid tumours. *Br. J. Clin. Pharmacol*. 2020. **86**: 1836–1848.

100 **Christian, B. A., Poi, M., Jones, J. A., Porcu, P., Maddocks, K., Flynn, J. M., Benson, D. M. et al.**, The combination of milatuzumab, a humanized anti-CD74 antibody, and veltuzumab, a humanized anti-CD20 antibody, demonstrates activity in patients with relapsed and refractory B-cell non-Hodgkin lymphoma. *Br. J. Haematol*. 2015. **169**: 701–710.

Abbreviations: **4-IPP**: 4-iodo-6-phenylpyrimidine · **25-HC**: 25-hydroxy-cholesterol · **AIF**: apoptosis-inducing factor · **AIS**: American Spinal Injury Association Impairment Scale · **Akt**: protein kinase B · **Bcl**: B cell lymphoma · **CCL**: CC-motif chemokine ligand · **CD74-ICD**: intracellular domain of CD74 · **CH25H**: cholesterol-25-hydroxylase · **COX2**: cyclooxygenase 2 · **CLL**: chronic lymphocytic leukemia · **CSB**: Chicago Sky Blue · **CXCR**: CXC-motif chemokine receptor · **DMTF1**: Cyclin-D-binding Myb-like transcription factor 1 · **DVT**: deep vein thrombosis · **ELISA**: enzyme-linked immunoassay · **ERK**: extracellular signal-regulated kinase · **IHC**: immunohistochemistry · **MAPK**: mitogen-activated protein kinase · **(r)MIF**: (recombinant) macrophage migration inhibitory factor · **mPGES-1**: microsomal PGE synthase-1 · **PARP-1**: poly(ADP-ribose) polymerase-1 · **PAX-5**: Paired Box 5 · **PGE_2**: prostaglandin E2 · **PI3K**: phosphoinositide 3-kinase · **(m/sh/si)RNA**: (messenger/short hairpin/small interfering) ribonucleic acid · **SCI**: spinal cord injury · **ZAP-70**: zeta-chain-associated protein kinase.

Full correspondence: Dr. Judith Fraussen, Department of Immunology and Infection, Biomedical Research Institute, Hasselt University, Martelarenlaan 42, Hasselt 3500, Belgium
e-mail: Judith.fraussen@uhasselt.be

Received: 27/6/2024
Revised: 7/10/2024
Accepted: 8/10/2024
Accepted article online: 13/10/2024