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Previews

Cytokine aerobics: Oxidation controls cytokine dynamics and function

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In this issue of *Structure*, Skeens et al. provide insights into the structure and dynamics of an oxidized form of the atypical cytokine macrophage migration-inhibitory factor (MIF). The study unveils a surprising conformational susceptibility of MIF to ambient redox alterations and identifies redox-sensitive residues and latent allostery sites with functional relevance.

Macrophage migration-inhibitory factor (MIF) is a multifunctional inflammatory cytokine and atypical chemokine (ACK). MIF is an important upstream modulator of the innate (and adaptive) host immune response, but, when deregulated, it promotes a variety of pathologies including acute and chronic inflammatory conditions, cardiovascular diseases, cancer, and autoimmunity (Calandra and Roger, 2003; Kang and Bucala, 2019; Schindler et al., 2018). The MIF protein is evolutionarily highly conserved with orthologs found in various kingdoms and species, including plants and unicellular parasites, suggesting that the MIF protein family has evolved 800 million years ago and that MIF proteins may have originally exerted non-cytokine intracellular functions. This may explain, at least in part, a number of the intriguing atypical molecular properties that have been identified for MIF and that differentiate it from conventional cytokines and chemokines (Schindler et al., 2018). To this end, the MIF sequence does not contain an N-terminal signal sequence, and translated MIF resides in the cytosolic and nuclear cellular compartment, from where it is released into the extracellular space by an unconventional secretion pathway. Extracellular MIF signals through its cognate receptor CD74 (a type II membrane protein that also functions as a major histocompatibility complex class II chaperone) to regulate a variety of immune and inflammatory activities as well as tumor cell proliferation (Figure 1). MIF also engages in chemokine mimicry to bind to the CXC-type chemokine receptors CXCR2, CXCR4, and CXCR7/ACKR3, a signaling network important in atherogenic leukocyte recruitment responses, tumor cell migration, and pulmonary fibrosis (Figure 1; Calandra and Roger, 2003; Kang and Bucala, 2019; Schindler et al., 2018). While MIF's α/β-containing structural fold is somewhat reminiscent of the overall folds of interleukin-(IL)-1β and chemokines such as IL-8/CXCL8 (Sun et al., 1996), and while its surface-exposed regions partially mimic binding motifs for the aforementioned chemokine receptors, the sequence homology to the corresponding classical chemokine ligands is limited and MIF proteins cannot be structurally classified into any of the known cytokine or chemokine sub-classes (Calandra and Roger, 2003; Schindler et al., 2018). In contrast, the three-dimensional architecture of the MIF trimer strikingly resembles that of bacterial tautomerases such as 4-oxalocrotonate tautomerase (4-OT) (Calandra and Roger, 2003; Sun et al., 1996). Moreover, MIF shares with these enzymes an N-terminal proline residue that forms the core of a conserved catalytic tautomerase pocket. Herein and in the *Structure* paper by Skeens and colleagues, this proline residue is termed Pro-1, but across the literature, it is also referred to as Pro-2 because the N-terminal methionine residue preceding Pro-2 in the gene-encoding DNA sequence is post-translationally removed in most cells. While modification of the pocket by mutations, inhibitors, or oxidation/dehydrogenation of Pro-1 modulates some of MIF's receptor and signaling activities and pathogenic effects (Calandra and Roger, 2003; Kang and Bucala, 2019; Schindler et al., 2018), MIF's tautomerase activity has so far only been demonstrated *in vitro*, and a substrate and physiological role in mammalian cells have yet to be identified, together insinuating that the tautomerase site mainly indirectly affects MIF's receptor-mediated cytokine activities. MIF's evolutionary connection also is a likely explanation for the conservation of a redox-active Cys-Xaa-Xaa-Cys (CXXC; Cys-Ala-Leu-Cys [CALC] in MIF) motif that it shares with thiol-protein oxidoreductases (TPORs) (Kleemann et al., 1998) and that has been linked to some of MIF's disease-related effects (Schindler et al., 2018). Of note, recent work by Schinagl and coworkers identified Cys-80 as a redox-sensitive conformational switch in MIF, triggering the conversion of reduced MIF (redMIF) to oxidized MIF (oxMIF) (Schinagl et al., 2018), similar to the findings of the current *Structure* article by Skeens et al. (2022). Furthermore, capitalizing on an oxMIF-specific antibody that specifically binds to an oxidized (disulfide) form of a CALC-spanning MIF peptide, they proposed a link between the switch residue cysteine 80, an unleashing of the redox-active CALC motif and pathogenic activities of MIF related to oxidative microenvironments (Schinagl et al., 2018). However, the structural and atomic details of the redox-dependent conformational switch remained elusive.

In the current issue of *Structure*, Skeens et al. (2022) use solution nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry to elucidate the

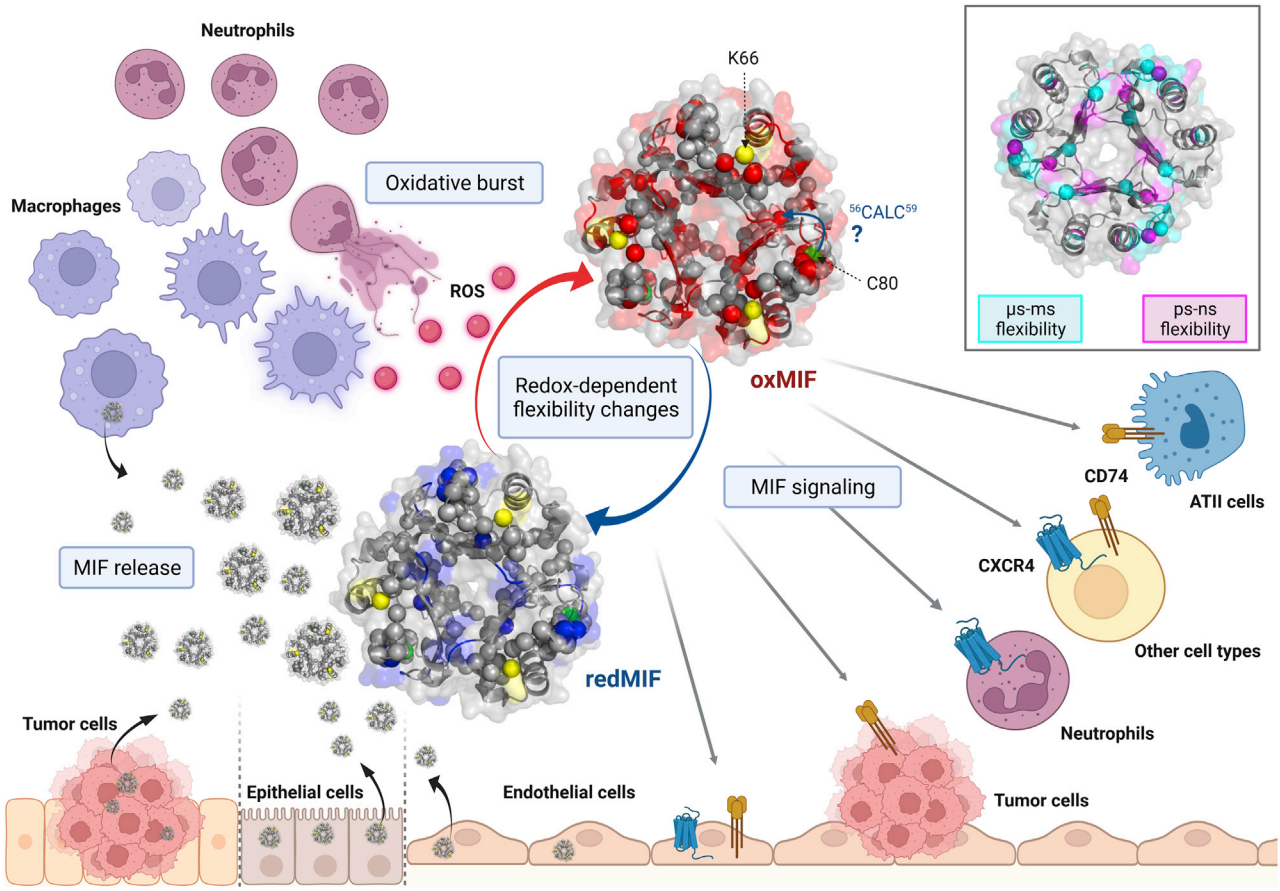

Figure 1. Structure and dynamics of MIF are altered by microenvironmental redox conditions with implications for MIF receptor interactions

MIF is known to be secreted by a variety of cell types, including macrophages, endothelial, and certain epithelial cells as well as tumor cells. Once secreted, MIF is exposed to the local microenvironmental redox potential and may, for example, be oxidized in an inflammatory context where both macrophages and neutrophils promote oxidizing conditions via an oxidative burst, creating reactive oxygen species (ROS) and other pro-oxidative mediators. Skeens and colleagues show that wide areas of the MIF molecule selectively become more flexible under oxidizing (or reducing) conditions, marked in either red (in the "oxMIF" conformation) or blue ("redMIF" conformation). Their data further suggest that residues Cys-80 (C80, green spheres) and Lys-66 (K66, yellow spheres) act as redox sensors or switches between both conformations. A role for C80 as redox switch for a conformational rearrangement of the ^{56}CALC^{59} motif of MIF, as previously suggested by Schinagl et al. (2018), is indicated as well. Residues of MIF that are involved in engaging its cognate receptor CD74 and its non-cognate receptors CXCR2 and CXCR4 are visualized as spheres. As these areas of MIF overlap significantly with residues changing their dynamics especially under oxidative conditions, becoming more flexible, this redox-dependent change in protein dynamics could have consequences for MIF's ability to signal through these receptors, which could be expressed on endothelial and tumor cells, but also on alveolar type II epithelial (ATII) cells, neutrophils, monocytes/macrophages, or other cell types. The upper-right inset highlights redox-sensitive residues of MIF that show either μs-ms (cyan) or ps-ns flexibility (magenta). Among these are residues I64, Y95 N97, N109, and S111 (depicted as spheres) that were shown previously to control CD74 activation and the enzymatic activity of MIF. The shown structures of MIF were adapted from the PDB entry *1MIF* structure using PyMOL version 1.8.2.2 (Schrödinger, LLC), and the figure was created with BioRender.com

structural details of such a redox-dependent conformational switch in MIF and to test the hypothesis that MIF may act as an allosteric "redox sensor" to respond to pro-oxidative inflammatory microenvironments as a means to fine-tune its downstream effector activities. With NMR spin relaxation experiments under oxidative versus redox-neutral and reducing solution conditions, and by employing site-specific mutagenesis of identified "latent" redox-sensitive residues, they (1) found that MIF is extensively modified in an oxidative environment, (2) found that it becomes remarkably dynamic under such conditions with motions observed in the μs-ms but also ps-ns timescale, (3) found that oxMIF exhibits the most pronounced conformational and dynamic changes, (4) determined MIF's redox potential to be in the near-to-physiological range (midpoint: $E'_0 = -215$ mV ± 15 mV), (5) discovered that residues Cys-80 and Lys-66 serve as redox-sensitive, latent allosteric sites to toggle MIF conformation and function, and (6) discovered that mutation of these switches ablates MIF's tautomerase activity and attenuates its inflammatory neutrophil recruitment activity in murine lungs *in vivo*. In conjunction, these results unveil previously unrecognized substantial oxidation-triggered changes in the conformation and dynamics of the MIF molecule and implicate these alterations as a molecular switch mechanism to fine-tune MIF effector functions.

There are commonalities between the 2018 study by Schinagl and colleagues and the current paper published in *Structure* (Schinagl et al., 2018; Skeens et al., 2022). Schinagl and coworkers also identified Cys-80 as a switch cysteine for the conversion from redMIF to oxMIF; implicated redMIF as a latent, inactive,

form of MIF; and suggested that formation of oxMIF leads to an isoform of MIF with certain pathophysiological characteristics (Schinagl et al., 2018). However, regarding MIF's effector activity, they solely focused on MIF's catalytic redox activity to suggest that an oxidation-dependent activation of MIF via Cys-80 leads to a conformational change and activation of the CALC redox motif. The mechanism, however, how CALC-mediated redox effects might affect MIF's downstream signaling activities has remained obscure. Skeens and colleagues did not study effects on CALC, also because the corresponding CALC-spanning peptide was not amenable to the mass spectrometry methods used in their study. Instead, they took a wholistic, impressively comprehensive look at the redox signature of MIF and its conformational, dynamic, and functional consequences. Most importantly, this provides us with detailed and unprecedented insight into the redox-dependent structure and dynamics of MIF at the molecular level. The dynamic measurements not only identified μs-ms dynamics predominant in oxMIF but also changes in the ps-ns timescale that can be observed in redMIF. In addition to Cys-80, they identified Lys-66 as a critical redox-sensitive residue. While Lys-66 is not covalently redox-modified itself, it undergoes large changes in flexibility between the oxidized and reduced states of MIF. Although it remains to be clarified how precisely this residue engages in and mediates redox-triggered conformational changes, as it is positioned at the bottom of the first α-helix with a supposed prime role in stabilizing the internal structure of the MIF fold, we suggest to term it a "secondary" redox switch residue. Importantly, in conjunction with their mutational experiments, Skeens and coworkers provide convincing evidence that Cys-80 and Lys-66 function as redox-sensitive latent allosteric sites (i.e., "switch" residues) for the generation of oxMIF that is accompanied by increased dynamics, refolding, and altered receptor binding properties. Thus, in contrast to the CALC redox concept suggested by Schinagl and colleagues, the current study in *Structure* suggests that the conversion to oxMIF unleashes receptor binding and/or activation sites in MIF (Skeens et al., 2022). The performed pulmonary neutrophil recruitment assay implies that this involves an activation of MIF/CD74 engagement (Figure 1), a notion that is in line with previous evidence on conformational dynamics and plasticity of MIF and a previously established connection between nanosecond dynamics of MIF and the MIF/CD74 binding interface (Pantouris et al., 2018), but this will have to be ultimately confirmed by direct evidence from receptor-binding and signaling experiments. Still, structural alterations in the MIF tautomerase pocket (i.e., by inhibitor binding or mutations) have previously been shown to lead to reductions in receptor activation by MIF, and MIF's tautomerase activity is influenced by an allosteric site at the central solvent channel also implicated in the current study by Skeens and colleagues (Pantouris et al., 2018, 2020; Skeens et al., 2022), lending further support to the redox-dependent latent allostery concept suggested here. Mechanistically, the switch residues might form covalent interactions with cysteine or lysine residues on CD74 that are proximal to the predicted interaction site between MIF and CD74, in line with previous suggestions (Meza-Romero et al., 2016). However, mechanisms of CD74 activation by redMIF have not been directly tested in the current study and at this point clearly cannot be excluded. Moreover, it is unlikely that CD74 is the only MIF receptor affected by redox modifications of MIF and altered dynamics in oxMIF. For example, CXCR4 is prominently expressed on the immune, endothelial, and parenchymal cells anticipated to populate disease-related pro-oxidative inflammatory microenvironments (Figure 1). Binding of MIF to this receptor also is affected by structural changes in MIF's tautomerase pocket, and CXCR4 contains potentially redox-susceptible cysteine residues as well as lysines in its ectodomain that may be amenable to redox-dependent interactions with the identified MIF switch residues.

Together, the paper by Skeens et al. (2022) published in this issue of *Structure* provides compelling evidence for a novel mechanistic paradigm of how microenvironmental redox conditions may influence MIF structure, dynamics, and function. Although several studies over the past two decades have established links between MIF and redox control in pathogenic conditions such as cancer, inflammatory diseases, and ischemia/reperfusion injury, partially addressing underlying structure-activity relationships, Skeens and colleagues for the first time provide a comprehensive picture of the redox signature of MIF at the molecular level. This adds an important facet to our understanding of how the MIF protein network may be regulated. Currently incompletely understood mechanistic questions, as to which MIF receptor is preferentially activated by extracellular MIF in a given disease, cell, or tissue context, may be partially governed by the specific redox signature of MIF. Oxidized MIF (or redMIF) may display a higher binding affinity or an enhanced EC_{50} value for a certain MIF receptor in a given redox microenvironment, thus paving the way for a certain signaling bias. Although the nuclear/cytosolic compartment generally exhibits a reducing redox potential, intracellular MIF protein-protein interactions might also be controlled by fluctuations in the cellular redox state.

The data obtained by Skeens et al. (2022) call for future studies to elucidate the structure-function relationships between specific redox signatures of MIF and its receptors (as well as its other binding partners) in more detail. Moreover, it will be interesting to explore if the MIF homolog MIF-2/D-DT or MIF orthologs such as plant-derived MIF/D-DT-like (MDL) proteins may have similar redox-sensitive conformational and dynamic properties as MIF. Lastly, the current study should aid in developing tailored therapeutic approaches with improved specificity and efficacy.

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DECLARATION OF INTERESTS

J.B. is an inventor on patent applications related to anti-MIF/anti-chemokine strategies in inflammatory and cardiovascular diseases. J.B. and M.B. are members of the m4 project team preclinical development of peptide-based chemokine receptor mimics as ligand-selective compounds for atherosclerotic diseases, a pre-seed competition award by the Bavarian State Ministry of Economic Affairs, State Development, and Energy.

REFERENCES

Calandra, T., and Roger, T. (2003). Macrophage migration inhibitory factor: a regulator of innate immunity. Nat. Rev. Immunol. *3*, 791–800. https://doi.org/10.1038/nri1200.

Kang, I., and Bucala, R. (2019). The immunobiology of MIF: function, genetics and prospects for precision medicine. Nat. Rev. Rheumatol. *15*, 427–437. https://doi.org/10.1038/s41584-019-0238-2.

Kleemann, R., Kapurniotu, A., Frank, R.W., Gessner, A., Mischke, R., Flieger, O., Jüttner, S., Brunner, H., and Bernhagen, J. (1998). Disulfide analysis reveals a role for macrophage migration inhibitory factor (MIF) as thiol-protein oxidoreductase. J. Mol. Biol. *280*, 85–102. https://doi.org/10.1006/jmbi.1998.1864.

Meza-Romero, R., Benedek, G., Leng, L., Bucala, R., and Vandenbark, A.A. (2016). Predicted structure of MIF/CD74 and RTL1000/CD74 complexes. Metab. Brain Dis. *31*, 249–255. https://doi.org/10.1007/s11011-016-9798-x.

Pantouris, G., Ho, J., Shah, D., Syed, M.A., Leng, L., Bhandari, V., Bucala, R., Batista, V.S., Loria, J.P., and Lolis, E.J. (2018). Nanosecond dynamics regulate the MIF-Induced activity of CD74. Angew. Chem. Int. Ed. Engl. *57*, 7116–7119. https://doi.org/10.1002/anie.201803191.

Pantouris, G., Khurana, L., Ma, A., Skeens, E., Reiss, K., Batista, V.S., Lisi, G.P., and Lolis, E.J. (2020). Regulation of MIF enzymatic activity by an allosteric site at the central solvent channel. Cell Chem. Biol. *27*, 740–750.e5. e745. https://doi.org/10.1016/j.chembiol.2020.05.001.

Schinagl, A., Kerschbaumer, R.J., Sabarth, N., Douillard, P., Scholz, P., Voelkel, D., Hollerweger, J.C., Goettig, P., Brandstetter, H., Scheiflinger, F., and Thiele, M. (2018). Role of the cysteine 81 residue of macrophage migration inhibitory factor as a molecular redox switch. Biochemistry *57*, 1523–1532. https://doi.org/10.1021/acs.biochem.7b01156.

Schindler, L., Dickerhof, N., Hampton, M.B., and Bernhagen, J. (2018). Post-translational regulation of macrophage migration inhibitory factor: Basis for functional fine-tuning. Redox Biol. *15*, 135–142. https://doi.org/10.1016/j.redox.2017.11.028.

Skeens, E., Gadzuk-Shea, M., Shah, D., Bhandari, V., Schweppe, D.K., Berlow, R.B., and Lisi, G.P. (2022). Redox-dependent structure and dynamics of macrophage migration inhibitory factor reveal sites of latent allostery. Structure *30*, 840–850. https://doi.org/10.1016/j.str.2022.03.007.

Sun, H.W., Bernhagen, J., Bucala, R., and Lolis, E. (1996). Crystal structure at 2.6-A resolution of human macrophage migration inhibitory factor. Proc. Natl. Acad. Sci. U S A. *93*, 5191–5196. https://doi.org/10.1073/pnas.93.11.5191.

A new twin expands the VirB8-like protein family

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Conjugative transfer is mediated by specialized type IV secretion systems (T4SSs). However, their architecture and mode of function remain poorly defined in Gram-positives. In this issue of *Structure*, Jäger et al. reveal an exclusive assembly of PrgL and illustrate the importance of its structural organization in pCF10 conjugative transfer.

The spread of antibiotic resistance is an expected outcome of bacterial adaptation to antimicrobial exposure. This phenomenon has been exacerbated in recent decades by the massive use of antibiotics in human and veterinary medicine, which has resulted in the emergence of multidrug-resistant bacteria, including many Gram-positive pathogens. Conjugative transfer is one of the main mechanisms driving the spread of antibiotic resistance genes among bacteria. This process is mainly carried out by mobile conjugative elements including plasmids and integrative and conjugative elements (ICEs). Their conjugative transfer is mediated by specialized type IV secretion systems (T4SSs) encoded by these elements.

While T4SSs have been well described in Gram-negative systems, there is a great lack of knowledge on their biogenesis in Gram-positives and how they ensure DNA transfer from the donor to recipient cell (Grohmann et al., 2018). There is a general consensus that T4SSs in Gram-positives adopt assembly and functional modes distinct from those found in Gram-negatives (Costa et al., 2021). The comparison of T4SSs gene clusters from different conjugative elements from Gram-positives revealed that T4SSs display a considerable variation in the composition of their transfer proteins. Nevertheless, most of the T4SSs gene clusters encode a minimal set of conserved proteins, which have been named according to their homologous VirB components of the Archetypal VirB/D4 T4SS from the Ti plasmid of *Agrobacterium tumefaciens*. A concept of minimal T4SS in Gram-positives was proposed earlier (Bhatty et al., 2013). This model suggests that T4SSs form a channel across the bacterial envelope, which is composed of two functional modules: the translocon/ATPase complex mediating DNA translocation across the cytoplasmic membrane and the cell wall complex allowing the passage of the DNA through the cell wall. This assembly model remains speculative. Further structural and functional analyses of T4SS transfer proteins are needed to better understand how Gram-positive T4SSs assemble within the bacterial envelope and how they ensure DNA transfer.

Among the structural components of T4SSs found in both Gram-negatives and Gram-positives, VirB8 proteins form a superfamily of proteins known to be important in the functioning of these nanomachines. However, the structural and molecular aspects of their involvement in T4SS assembly and function are still poorly understood. During the last decade, several studies have been focused on the structural and functional characterization of VirB8 from Gram-positives (referred to as VirB8-like) (Cappele et al., 2021; Fercher et al., 2016; Goessweiner-Mohr et al., 2013; Porter et al., 2012). Together, these studies emphasized the importance of