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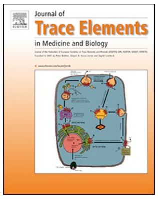

Nutrition

Alterations in membrane fluidity are involved in inhibition of GM-CSF-induced signaling in myeloid cells by zinc

Isabell Aster, Lisa-Marie Barth, Lothar Rink, Inga Wessels*

Institute of Immunology, Medical Faculty, RWTH Aachen University, Pauwelsstr. 30, D-52074 Aachen, Germany

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ABSTRACT

Zinc has a strong influence on the function of the immune system and is a driving factor for immune cell development. In this regard, studies revealed cell type specific effects of zinc. During zinc deficiency for example, development and activity of myeloid cells seems to be prioritized at the cost of cells from the lymphoid lineage. In T-cells, the altered proliferation was found to be due to zinc's effect on IL-2-induced signaling processes, but in contrast to lymphoid cells, effects of zinc homeostasis on growth-factor-induced signaling in myeloid cells have not been investigated yet. The granulocyte-macrophage colony-stimulating factor (GM-CSF) is one of the major factors inducing monopoiesis. Considering the structural similarities between the GM-CSF receptor and those of the IL-receptor family as well as a similar set of signaling molecules involved, an impact of zinc on the GM-CSF signaling seems to be likely.

Therefore, the effect of zinc on GM-CSF-induced signaling molecules was investigated here, using U937 cells as a model myeloid cell line.

GM-CSF stimulation significantly increased STAT5 phosphorylation which was prevented completely by pre-incubation with zinc and pyrithione. U937 cells showed a strong pre-activation regarding c-Raf, which was significantly decreased by zinc and pyrithione incubation, independently from GM-CSF stimulation.

As current literature was not sufficient to explain the observed effects, we hypothesized an altered receptor-complex assembly. As membrane composition and plasticity, subsumed under the term of membrane fluidity, was found to affect receptor multimerization, the impact of zinc on membrane fluidity was considered as a completely novel approach. Indeed, addition of zinc also decreased GM-CSFR expression on the cell surface and most interestingly altered membrane fluidity.

In conclusion, we hypothesize that the incubation with zinc causes an alteration of membrane fluidity that hinders efficient receptor assembly as well as phosphorylation of signal molecules and therefore signal transduction.

1. Introduction

Zinc has a strong influence on the development and function of the immune system and is thus an established "gatekeeper of immune function" [1,2]. Monopoiesis is, for example, augmented during zinc deficiency while lymphopoiesis seems to be decreased [3]. Also, studies revealed cell type specific effects of zinc on immune function, such as T-cell activation and signaling which benefit from zinc supplementation [4].

Moreover, zinc affects basic functions of monocytes, macrophages and granulocytes such as phagocytosis and oxidative burst which are increased under zinc-deficient conditions [5,6]. This offers one explanation for the increased number and reactivity of myeloid cells during infection, when zinc is transiently transferred to the liver, causing serum hypozincemia [7]. However, multiple questions in regard to mechanisms underlying the zinc induced alterations in myelopoiesis are not clearly answered, yet.

A major factor balancing the development of the innate immune system is the granulocyte-macrophage colony-stimulating factor (GM-CSF), a haematopoietic growth factor with a major influence on survival, differentiation and activity of monocytes. It commits primitive progenitor cells to their lineage, but also targets at mature cells [8] affecting their chemotaxis and adhesion to allow the identification of and access to the infected tissue [9,10].

All these effects are transmitted by the GM-CSF-receptor (GM-CSFR). It consists of one alpha- and one beta-chain, forming a dimer

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* Corresponding author.
E-mail address: iwessels@ukaachen.de (I. Wessels).

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upon GM-CSF binding [11]. As the GM-CSFR does not have an intrinsic tyrosine kinase activity, signaling is transmitted *via* recruitment of the tyrosine Janus kinase (Jak)2 which phosphorylates Src. Further signaling is transduced *via* Signal Transducer and Activator of Transcription (STAT)5 and c-Raf among others [12]. Substrate binding also induces GM-CSFR endocytosis, but involvement in signal transduction is unclear [13,14].

The importance of zinc for cellular signalling was amongst others shown for interleukin (IL)-2R signaling in T-cells [4,6]. MAPK cascade, especially ERK1/2 activation, plays a central role within this pathway. Dephosphorylation by protein phosphatase 2A and dual specificity protein phosphatases terminates signaling. Kaltenberg et al. showed that with addition of zinc, dephosphorylation was inhibited and ERK signaling could thereby be maintained whereas STAT5 signaling was not altered significantly. Moreover, zinc deficiency increased phosphorylation of c-Raf and augmented c-Raf and STAT5 phosphorylation induced by IL-2 [15]. Interestingly, a similar set of signaling molecules is involved in GM-CSFR-mediated signaling. Considering the structural similarities between this receptor and those of the IL-2 receptor family, an impact of zinc on the GM-CSF signaling seems to be likely. Therefore, our aim here was to examine the association between zinc homeostasis and GM-CSF-induced signaling, to address a possible explanation for changes in myelopoiesis that were observed during zinc deficiency [3].

2. Methods and materials

2.1. Cell culture and incubation with zinc and pyrithione

Pro-myeloid human U937 cells were incubated in RPMI-1640 (Sigma-Aldrich, St. Louis, Missouri, USA) containing 10% heat inactivated FCS, 2 mM L-glutamine, 100 U/ml potassium penicillin and 100 µg/ml streptomycin sulfate (Sigma-Aldrich) at 37 °C in a humidified 5% CO_2 atmosphere. For all performed assays, 1×10^6 cells/ml were incubated either without additional treatment (Medium, Med) or 100 µM zinc sulfate (Invitrogen, Thermo Fisher Scientific, Waltham, Massachusetts, USA) and 50 µM sodium pyrithione (Sigma-Aldrich), or 50 µM $CuSO_4$, $CaCl_2$, $MgCl_2$ (all Sigma-Aldrich) were added for 30 min and subsequently stimulated with GM-CSF (50 U/ml) (Thermo Fisher Scientific) for another 30 min at 37 °C.

2.2. Flow cytometry

U937 cells were stimulated with GM-CSF (50 U/ml) (Thermo Fisher Scientific) as described earlier. Flow cytometric data were recorded on a FACScalibur (BD Biosciences, Heidelberg, Germany) using Cellquest software 3.0 with a total of 20,000 events being recorded for each sample, using a gating strategy as described in Supplemental Fig. S1. Free intracellular zinc was assessed using FluoZin3-AM (1 µM) (Invitrogen) as described previously [16]. For measurement of the expression of CD116, stimulated cells were labelled with PE-coupled anti-human CD116 antibody (BioLegend, Koblenz, Germany) or respectively isotype control (PE mouse IgG1 κ, BioLegend).

2.3. Atomic absorption spectroscopy (AAS)

Untreated control cells were investigated compared to cells stimulated using GM-CSF (50 U/ml) or zinc sulfate (100 µM) and sodium pyrithione (50 µM). The cells were incubated for up to 1 h at 37 °C. The cells were then counted, and 1×10^6 cells/ml of every assay were suspended in 20 µl of water, digested using 200 µl of 70% HNO_3 for 3 h at 85 °C and the final volume was adjusted to 1 ml with water. Total cellular zinc was measured *via* flame atomic absorption spectrometry (AAS, Perkin Elmer, Baesweiler, Germany) as described previously [17].

2.4. Reverse transcription and real-time PCR

RNA was isolated using the ExtractME® RNA Kit (blirt, 7bioScience, Freiburg, Germany) and cDNA was acquired through use of qScriptTM cDNA Synthesis Kit (Quanta Biosciences, Darmstadt, Germany) according to the manufacturers' protocols. The primers for CD116 and the primers for the housekeeping gene glycerinaldehyde 3-phosphate dehydrogenase (GAPDH) were used as previously described [18,19]. All samples were run using a Step-1 plus (Applied Biosystems, Darmstadt, Germany) with the following parameters: 95 °C for 15 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min for GAPDH and 95 °C for 10 min, followed by 40 cycles of 95 °C for 45 s and 72 °C for 45 s for CD116 using Power SYBR Green PCR Master Mix (Applied Biosystems). Fold-changes were calculated through the ΔΔ CT method.

2.5. Western blotting

U937 cells were starved overnight in medium containing 2% FCS. After incubation as described above, 1×10^6/ml cells were lysed in sample buffer (65 mM Tris−HCl, 25% glycerol, 2% SDS, 0.01% bromophenol blue, 1% β-mercaptoethanol and 1 mM sodium orthovanadate), sonicated for 30 s and subsequently boiled at 95 °C for 5 min. The lysates were separated electrophoretically on 10% polyacrylamide gels and transferred onto a nitrocellulose membrane (BioRad, Munich, Germany). Membranes were blocked with 5% fat-free dry milk in TBS-T (20 mM Tris−HCl, 136 mM NaCl, 0.1% Tween 20) for 1 h. The phosphorylation state of STAT5 and c-Raf was analysed using primary antibodies against total STAT5, phosphorylated STAT5 (Tyr694), Raf and phosphorylated Raf (p-c-Raf338) (all obtained from New England Biolabs, Germany). Primary antibody incubation was performed over night at 4 °C with constant shaking. After 3 times of washing with TBS-T, membranes were incubated with a secondary antibody (HRP-coupled anti-rabbit IgG and HRP-coupled anti-biotin antibody, Cell Signaling Technology, Frankfurt, Germany) for at least 5 h at room temperature. Using LumiGlo reagent (Cell Signaling Technology) bands were detected on a LAS 3000 (Fujifilm Lifesciences, Düsseldorf, Germany). Resulting bands were quantified using ImageJ (ImageJ 1.46r, Wayne Rasband, National Institutes of Health, USA).

2.6. Fluidity measurement

Cells were incubated overnight in medium containing 2% FCS. First, the indicated supplements were added, and incubation was started. Then, the medium was supplemented with 0.08% Pluronic (P)F127 (Invitrogen, Thermo Fisher Scientific) and stained using 1-Pyrendecanoidacid (5 µM) (Invitrogen) and incubated with constant shaking for 20 min at room temperature excluding light. The cells were washed once with PBS containing PF127 and afterwards, fluorescence was measured on a Spark multimode reader (350/470 nm and 350/405 nm) (Tecan, Switzerland). Unstained cells were used and subtracted as blank and fluidity was calculated as the ratio of pyrene dimers to monomers.

2.7. Statistics

Statistic analysis was performed by a paired Student's *t*-test using Graph Pad Prism software (version 5.01, Graph Pad software, La Jolla, CA, UA). For multiple comparisons one-way Anova was used together with Tukey's post-hoc test. A p-value < 0.05 was considered to be statistically significant for both tests.

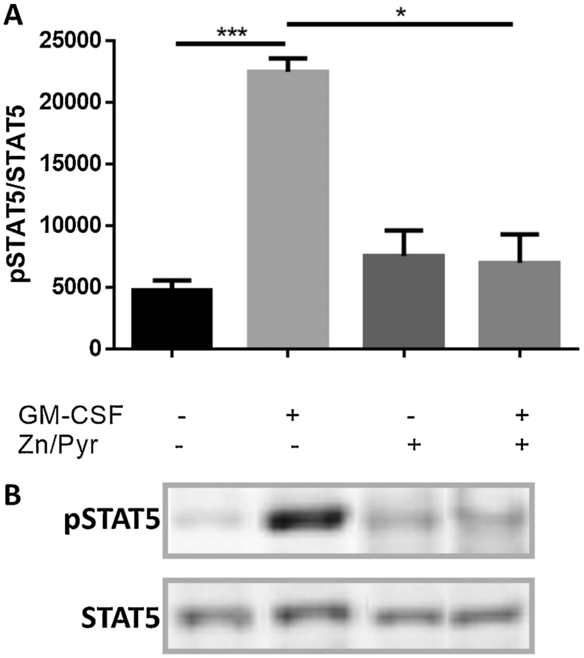

Fig. 1. Pre-incubation with zinc and pyrithione reduces GM-CSF-induced STAT5 activation. Pro-myeloid U937 cells were pre-incubated with zinc (Zn, 100 µM) and pyrithione (Pyr, 50 µM) for 30 min and subsequently stimulated with GM-CSF (50 U/ml) for another 30 min. Quantification was performed *via* optical density determination of n = 3 blots (A). Shown is one representative Western Blot (B). Results are presented as mean + SEM. Statistical significances, calculated by *t*-test, are depicted (*, $p < 0.05$; ***, $p < 0.001$).

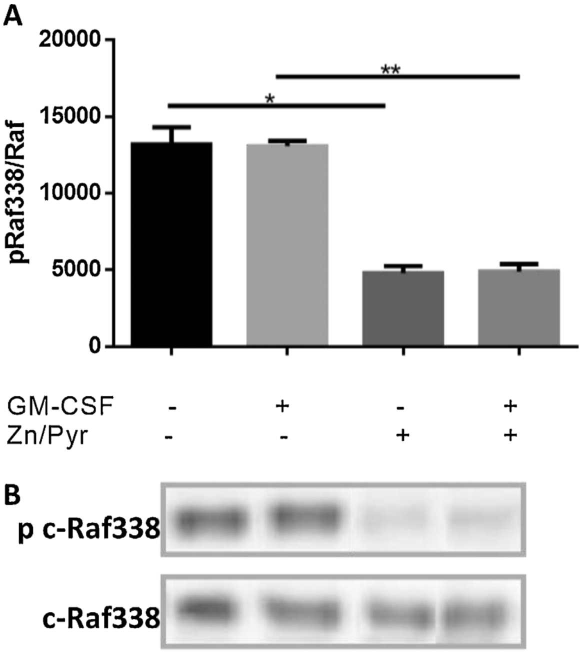

Fig. 2. Incubation with zinc and pyrithione decreases c-Raf phosphorylation. Effects of pre-incubation with zinc (Zn, 100 µM) and pyrithione (50 µM) on GM-CSF-induced c-Raf phosphorylation were investigated using Western Blots. (A) shows means + SEM for quantification of n = 4 Western Blots and (B) one representative example. *, $p < 0.05$, **, $p < 0.001$, student's *t*-test.

3. Results

3.1. Zinc affects GM-CSF-induced signaling

To analyse GM-CSF receptor-mediated signaling, U937 cells were stimulated with GM-CSF. Phosphorylated and unphosphorylated STAT5 as well as unphosphorylated c-Raf kinase and phosphorylated c-Raf kinase were measured *via* Western Blot. In unstimulated cells, STAT5 could predominantly be found in its unphosphorylated state. Addition of GM-CSF led to a four times higher phosphorylation of the molecule. Pre-incubation with zinc and pyrithione for 30 min did not initiate phosphorylation. Interestingly, zinc and pyrithione pre-incubation blocked GM-CSF-induced signaling (Fig. 1). Other signaling molecules such as STAT3 were not associated with GM-CSF signaling in this particular cellular model (data not shown).

On the other hand, c-Raf, part of the MAPK cascade, was already phosphorylated in the absence of GM-CSF. Upon stimulation with GM-CSF, no significant activation was achieved. Surprisingly, pre-incubation with zinc and pyrithione significantly reduced cRaf phosphorylation to less than 50% of the control levels, which was independent of the stimulation status of the cells in regard to GM-CSF (Fig. 2).

3.2. Intracellular zinc homeostasis is altered by GM-CSF stimulation

In order to further investigate the impact of GM-CSF on the cell's zinc homeostasis, intracellular free zinc was measured before and shortly after stimulation with GM-CSF using flow cytometry. 5 min after the addition of GM-CSF, a decrease of free zinc was detected (Fig. 3A). After another 5 min, the free zinc reached its former level.

To find out whether the stimulation with GM-CSF could also provoke effects on the total cellular zinc content, zinc was measured using atomic absorption spectroscopy (AAS). For up to 1 h, cells where either incubated with or without zinc and pyrithione or GM-CSF. In contrast to results for intracellular zinc (Fig. 3A), no effect of GM-CSF on total zinc was detected, whereas incubation with zinc and pyrithione resulted in a significant, more than four-fold increase in total zinc as early as 5 min after incubation (Fig. 3B).

3.3. Effects of zinc on membrane fluidity and receptor expression

As indicated earlier, GM-CSF receptor multimerization is required to induce signal transduction. A necessity of endocytosis of the receptor complex is discussed as well. Membrane fluidity, affected by membrane composition such as the cholesterol content and types of fatty acids and its plasticity, is crucial to the cell's capability to form multimers, for endocytosis and for recycling of receptors after ligand binding. Thus, we focussed our next experimental steps on analysis of membrane fluidity and receptor expression. U937 cells were stimulated with either zinc or a combination of zinc and pyrithione. To determine whether the interaction was time- or dose-dependent, several settings were tested (Fig. 4A).

Measurement of the fluidity *via* a multimode reader was performed. First, we observed that supplementation with zinc alone led to an altered fluidity over time compared to control, which was significantly different after 3 h of incubation. Addition of zinc together with pyrithione resulted in an even more impressive and significantly faster alteration of membrane fluidity (Fig. 4A). To exclude that the changes in membrane fluidity are a general phenomenon, when divalent ions are added, we repeated the experiment and added copper, magnesium or calcium in comparison to zinc. As U937 appeared sensitive to copper regarding vitality, 50 µM were chosen for all supplements. As depicted in Fig. 4B, only zinc caused significant changes in membrane fluidity, while copper, magnesium and calcium had no effect. Interestingly, a repetition of the experiment in a further differentiated myeloid cell line, THP-1 cells, revealed comparable results emphasizing the significance

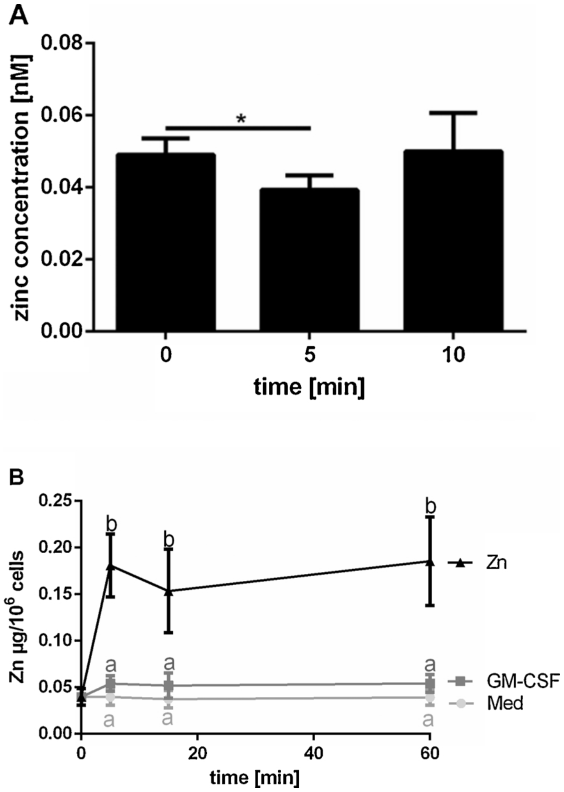

Fig. 3. GM-CSF causes an intracellular zinc flux but does not alter total cellular zinc (A) Intracellular free zinc in U937 was detected using the fluorescent probe FluoZin3-AM (1 μM) (n = 5). Results were compared to GM-CSF stimulated samples (50 U/ml). Values are shown as means + SEM, * indicates significant differences ($p < 0.05$, student's *t*-test). (B) Total zinc of U937 was detected using the AAS (n = 6). Means ± SEM for U937 incubated with zinc (Zn, 100 μM) combined with pyrithione (50 μM), GM-CSF (50 U/ml) or medium are shown for the indicated time points. Significantly different data sets do not share the same letter (*, $p < 0.05$, one-way Anova, Tukey's post-hoc test).

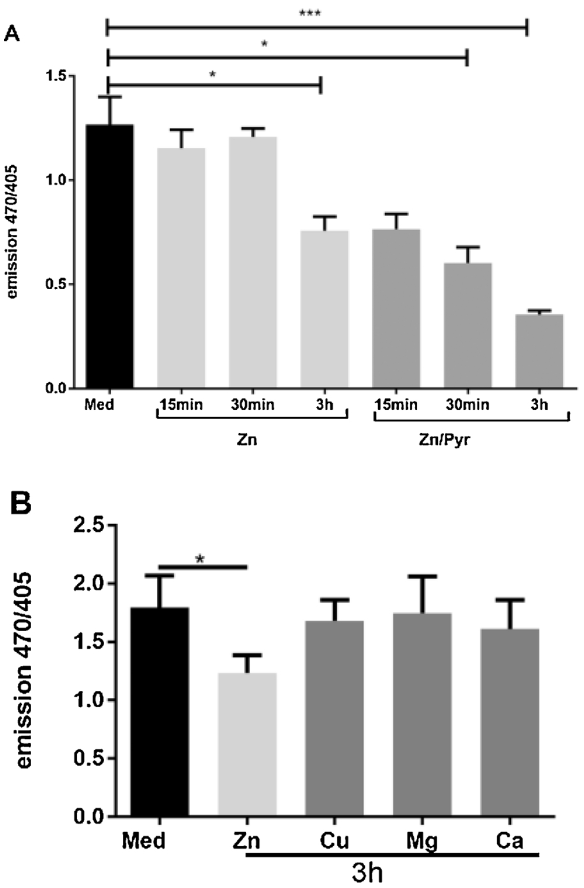

Fig. 4. Incubation with zinc altered membrane fluidity. U937 cells were starved in medium containing 2% FCS overnight. Stimulation was performed as indicated with zinc (Zn, 100 μM) or zinc (100 μM) and pyrithione (Pyr, 50 μM) (A) or with $ZnSO_4$ (50 μM), $MgCl_2$ (50 μM), $CaCl_2$ (50 μM) or $CuSO_4$ (50 μM) (B). Staining was done using medium with 0.08% Pluronic F127 and 1-pyrendecanoidacid (5 μM) for 20 min at room temperature excluding light. Fluorescence was measured on a Spark multimode reader (n = 3–4). Values are shown as mean + SEM, statistical significances, calculated by *t*-test, are indicated (*, $p < 0.05$; ***, $p < 0.001$).

of the observed effect for myeloid cells, while no significant changes were found for the lung cell line A549 (data not shown).

As mentioned earlier, receptor endocytosis might play a vital role in GM-CSF-induced signal transduction. To test if zinc plays a role in this process, cells where either kept in normal medium or were pre-incubated with zinc together with pyrithione and subsequently stimulated with GM-CSF for up to 30 min. Afterwards, flow cytometry was used to measure CD116, representing the expression of the receptor's alpha unit on the cell surface. In cells without zinc pre-incubation, CD116 expression was unaltered in reaction to GM-CSF stimulation, but constantly decreased when zinc and pyrithione were added (Fig. 5A). During prolonged incubation of the cells with zinc and pyrithione (Fig. 5B), CD116 levels further decreased, which was not altered, when GM-CSF was added 30 min after zinc and pyrithione pre-incubation had started (Fig. 5B). In contrast to GM-CSFR surface levels, *de novo* transcription of GM-CSFR mRNA was not affected by zinc and pyrithione incubation (data not shown).

4. Discussion

Zinc influences both the development and activity of myeloid cells, but underlying mechanisms have not been completely elucidated, yet. In this study, we examined the hypothesis that an alteration of GM-CSF signaling by zinc might be one of the underlying mechanisms. The GM-CSFR forms a multimeric complex upon GM-CSF binding [11] and signaling is transmitted by the tyrosine kinase Jak2, resulting in phosphorylation of STAT5 and c-Raf [12]. The importance of receptor internalization for GM-CSF-induced signaling is of doubt [13,14], which can be concluded from our results as well.

A first hint on the association of zinc and GM-CSF-induced signaling was that GM-CSF provoked a temporal decline of intracellular zinc. As total cellular zinc was unaltered, zinc is probably transiently redistributed within the cell, which has been reported and connected to the regulation of signal transduction before [20]. However, most other stimuli, including lipopolysaccharide, insulin or IgE receptor ligands, cause a transient increase in intracellular zinc. Thus, in regard to zinc homeostasis, GM-CSF seems to activate different mechanisms [21,22].

As leukemic cell lines do not necessarily respond to stimuli in the way primary cells do, we subsequently verified that signaling pathways were up-regulated by GM-CSF in our experimental setting, *i.e.* in U937 cells. We confirmed that GM-CSF stimulation significantly increases STAT5 phosphorylation. U937 cells showed a strong pre-activation regarding c-Raf, but no further activation after GM-CSF stimulation. This pre-activated state could be explicable by the origin of U937 cells which are derived from a leukaemia patient. In cases of acute leukaemia,

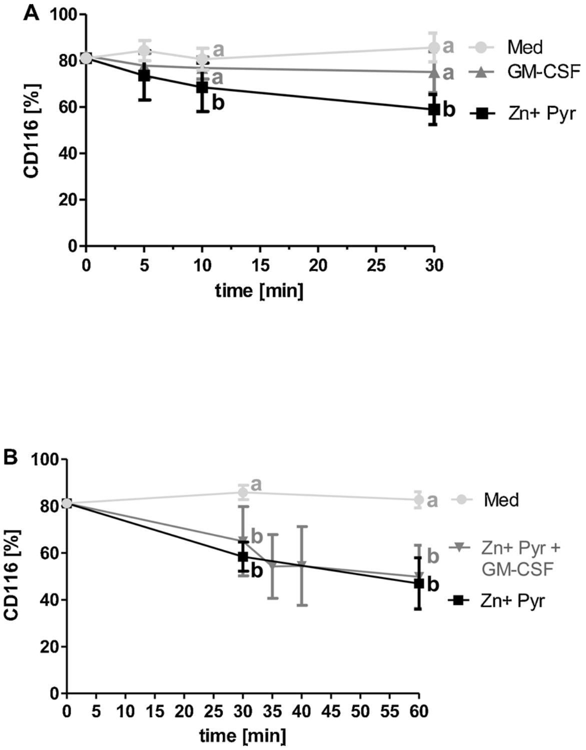

Fig. 5. Zinc and GM-CSF differentially effect GM-CSFR surface expression. Expression of CD116 on the surface of U937 cells was detected using flow cytometry at the indicated time points. (A) Cells were incubated with or without zinc (100 µM) and pyrithione (50 µM) or GM-CSF (50 U/ml) for up to 30 min. (B) U937 were pre-incubated with or without zinc (100 µM) and pyrithione (50 µM) for 30 min. Subsequently, GM-CSF (50 U/ml) was added for up to 30 min. The unstimulated control, without any treatment, is included as well. Results are presented as means ± SEM (n = 3). Data sets significantly different from each other do not share the same letter (one-way Anova and Tukey´s post-hoc test ($p < 0.05$).

activation and termination of the Ras cascade by Raf kinase inhibitor protein (RKIP) have already been shown to be impaired. This alteration stops signal transmission between c-Raf and MEK thereby terminating signal transduction [23,24]. In cells without RKIP activation, proliferation and colony formation were decreased [23]. Therefore, decoupling of Ras activation of the underlying growth factor stimulation can be assumed, explaining our finding. In our setting, both STAT5 as well as c-Raf were found in their unphosphorylated state after incubation with zinc and pyrithione. Literature shows examples for the zinc-induced inhibition of protein tyrosine phosphatases (PTPs) in a dose-dependent manner [25]. In T-cells, the phosphatase PTEN was described to be inhibited by high zinc conditions [15,26]. However, an inhibition of phosphatases would rather result in increased phosphorylation of STAT5, which contradicts our findings and suggests a cell type- and stimulus-specific effect of zinc in regard to signaling. Thus, alternative mechanisms needed to be found. STAT5 is phosphorylated by Jak2 after recruitment to the receptor complex. Studies on the effects of zinc on kinase activity are rare and no study on the effect on Jak2 activity in particular was found. Also, those existing studies indicate rather an activation of kinase activity by zinc, which contradicts our finding in regard to the phosphorylation of signaling pathways as well [25].

An alternative scenario might be a direct interaction of zinc with the signaling molecules involved. Several studies have proven an inhibition of Ras as well as STAT signaling under high zinc conditions [27–29]. In nematodes, high cytosolic zinc seemed to maintain Ras in an inactive state [27]. In $CD4^+$ cells, STAT3 activation was prevented by zinc treatment [28]. The authors hypothesized that an alteration of the 3D structure might be the explanation for this effect. They performed CD spectroscopy and confirmed an unfolding of STAT3 by zinc [28]. In their experiments, Jirakulaporn and Muslin searched for an explanation for the altered Ras activity in *C. elegans* under high zinc conditions. They found a zinc exporter, CDF-1 that supported both activation and translocation of Raf-1. CDF-1 lowered the intracellular zinc content as a precondition for signaling. In cells lacking the CDF protein, Ras-Raf-signaling was completely blocked [30]. In view of our experiments comparable connections are likely.

As we observed a very strong effect on GM-CSF signaling nearly blocking signaling completely, we assumed the effect to take place very early in the signaling cascade. The earliest event of course is receptor activation and receptor multimerisation. Therefore, we investigated this event in greater detail. As the formation of the GM-CSFR requires a re-arrangement of the subunits involved, we hypothesized that a modified membrane composition might be the key to the puzzle. Until now, the impact of zinc on membrane fluidity has not been considered for receptor integrity and therefore cell signaling. Our experiments represent the first attempt to enlighten this most interesting interaction. After treatment with zinc alone, we observed an altered fluidity compared to control. The addition of zinc and pyrithione in combination allows us to provoke a rapid and even stronger rise of the intracellular zinc which was obviously able to alter membrane fluidity to an even greater extent.

Both receptor activation with formation of a dodecamer and receptor endocytosis necessary for receptor recycling depend on a high grade of membrane flexibility which is only granted by membrane fluidity. Here, we provide evidence for changes in fluidity in reaction to zinc incubation, which might be responsible for alterations in receptor expression, multimerisation or endocytosis. Interestingly, phagocytosis, also depending on the membrane fluidity status, is already known to depend on a balanced zinc homeostasis, highlighting the likeliness of a causal relationship. In absence of zinc, a stronger phagocytosis could be detected [5].

As a first hint that alteration of membrane fluidity by zinc affects receptor assembly, analysis of CD116 levels on the surface of U937 cells revealed, that CD116 surface levels were constantly decreasing when zinc and pyrithione were added. This decrease was not altered when GM-CSF was added to the cells. Further analyses are required to find the exact mechanisms responsible here.

The influence of trace elements on cellular membrane composition is an interesting topic that remains for the most part unclear. García et al. made an attempt to illuminate the influence of metal ions in this regard [31]. Lipid fluidity is already known to be influenced by temperature as well as membrane composition, especially the cholesterol content or the amount of unsaturated of fatty acids. On the other hand, there is evidence of iron, chromium and copper causing alterations of the membrane structure [31,32]. Unfortunately, little is known about the underlying mechanisms. Verstraeten et al. showed an altered lipid composition after zinc incubation. Their results pointed to an increased translocation of phosphatidylserine as well as an altered composition of fatty acids [33]. Meta-analysis revealed an effect of zinc supplementation on the cholesterol homeostasis. The reaction observed differed between healthy and unhealthy individuals, but revealed significant modifications of HDL and LDL cholesterol [34]. Regarding the fact that cholesterol is a major component of plasma membranes, this could be an explanation for zinc-altered fluidity *in vivo*, but does not explain our *in vitro* observations. Furthermore, the interaction of the plasma membrane with the cellular cytoskeleton was shown to affect membrane rigidity [35]. The effect of zinc on the cytoskeleton is not completely understood, but it is known that high doses of zinc reduce microtubular function in neuronal cells [36]. In kidney cells, zinc was shown to impair the actin filament system [37] which is fundamental for endocytosis and degranulation of mast cells [38]. Considering the effect of zinc on phagocytosis discussed earlier, an interaction of zinc with the cytoskeletal components might contribute to the altered GM-CSF signaling observed here and should be taken into consideration in future studies. Nevertheless, other modes of interaction could be possible. Thus, a certain degree of membrane fluidity seems to be important for regular receptor functioning. Alterations such as those caused by incubation with zinc might be the explanation for altered surface expression of CD116 as well as altered formation of the receptor complex. Regarding our results, the influence of zinc-altered membrane fluidity on other receptor types is of high interest and should be the subject of further studies.

5. Conclusion

Zinc is involved in the regulation of GM-CSF-induced signaling, especially in activation of STAT5-mediated pathways, which might explain changes in monopoiesis during disturbed zinc homeostasis. Notwithstanding the similarities between IL2R- and GM-CSFR-mediated signaling, zinc seems to play different roles in both pathways. This underlines the diverse requirements of the innate and the adaptive immune system concerning the zinc homeostasis. There is evidence indicating the importance of the cellular zinc content for membrane fluidity. Our results suggest an influence of the altered membrane fluidity on GM-CSF-receptor function thereby modulating the receptor signaling. Currently, GM-CSF, or Sargramostim, is in clinical use for treatment of neutropenia after stem cell transplantation. More than 20 million people worldwide have been treated with Sargramostim [39]. A rapid recovery from this condition is crucial for the success of the treatment and the survival of the patient. Taken our results into account, the individual zinc status should be taken into consideration for optimization of the therapy of diseases that involve disturbed myeloid cell´s development or function. However, further research is needed to put this from bench to bedside.

Conflict of interest

None.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jtemb.2019.04.018.

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Key publications

Lienau et al., The role of zinc in calprotectin expression in human myeloid cells. JTEMB 49, 106–112, 2018, doi:https://doi.org/10.1016/j.jtemb.2018.04.022 .

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Inga Weßels: Education and Employment: Inga Weßels received her M. Sc. in Biotechnology in 2007 and her PhD in immunology in 2012, both from the RWTH Aachen University. From 2012–2015 she was a post-doctoral fellow in the Department of Food Science and Human Nutrition at the University of Florida in Gainesville, USA, where she worked with Professor R. J. Cousins. Since 2015, she is a PI in the Institute of Immunology at RWTH Aachen University Hospital, Germany, led by Professor L. Rink and has currently over ten years of experience in zinc research. **Research interests:** Key aspect of her work is the nutritional immunology, especially regarding zinc in the development and function of innate immune cells. Projects focus on establishing treatment strategies to prevent and treat inflammatory diseases as was recently investigated for sepsis and acute lung injury. In addition to human *in vitro* models, she includes *in vivo* and *in vitro* studies for mice, fish, chicken and pigs. Dr. Wessels is guest editor for the Journal of Trace Elements and Minerals in Biology and reviewer for several journals.