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Title: MIF Modification and the Regulation of Inflammation

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Introduction:

Macrophage migration inhibitory factor (MIF) is a protein involved in the immune system. If you scrape your knee, MIF is released from damaged cells, binds to its specific receptor called CD74 and helps maintain an immune response by encouraging inflammatory molecules to the site. Over-expression of MIF has been shown to lead to chronic inflammation in numerous diseases. MIF levels have been shown to correlate with the severity and invasiveness of several cancers, with over-expression increasing chance of both tumour growth and metastasis. Other diseases, such as heart disease and cystic fibrosis, have been associated with increased MIF serum levels. Clearly being able to inhibit MIF when it reaches certain concentrations could have numerous consequences for disease research.

As well as binding to CD74, MIF also has an enzyme activity. It seems the N-terminal proline residue is responsible for this activity, while it is also involved in recognition binding to the receptor. An important part of the immune response are white blood cells called neutrophils. They engulf and kill invading bacteria. In order to do this, neutrophils produce HOCl (bleach) which is a strong oxidant. We speculate that HOCl modifies MIF, altering the inflammatory response.

Aims:

The overall goal of this research is to determine if MIF is modified by HOCl in vivo. In order to achieve this, we would like to use a tagged form of MIF called HisMIF, which will enable us to pull MIF out of biological fluids and examine it.

Methods:

To confirm that HisMIF is modified by HOCl in a similar fashion to MIF, both types were placed several different concentrations of HOCl. This was to test how much HOCl was required to inhibit both MIF and HisMIF functioning. We then repeated this experiment, this time using a fluorescent compound called FITC that is known to also inhibit MIF function. MIF modification can be measured by determining the amount of fluorescence incorporated into the protein. A mass spectrometer was used to measure MIF modification.

Results:

HOCl and FITC were effective at inhibiting both MIF and HisMIF at the same concentration, therefore HisMIF will be useful in biological samples. When we measured the mass of these products, we found that only one molecule of FITC was bound per protein. This indicated that it was only bound at the N-terminal proline. MIF was also exposed to HOCl first, and then incubated with FITC. In this experiment, there was no evidence that the fluorescent compound could bind. This confirmed that FITC could only bind to the N-terminal proline, which was modified when exposed to HOCl first.

Conclusion:

We have demonstrated that MIF reacts in the same way as our modified MIF, HisMIF, in the presence of both HOCl (oxidant) and FITC (tautomerase inhibitor). Therefore, HisMIF can confidently be used and the findings applied to MIF. The host lab now intends to introduce tagged MIF in complex biological fluids with activated neutrophils to determine if modification occurs.