Supplementary data

Methylglyoxal-Modified Human Serum Albumin Binds to Leukocyte Myeloperoxidase and Inhibits its Enzymatic Activity

Oleg M. Panasenko [1,2,*], Viktor A. Ivanov [1], Elena V. Mikhalchik [1], Irina V. Gorudko [3], Daria V. Grigorieva [3], Liliya Yu. Basyreva [1], Ekaterina V. Shmeleva [1], Sergey A. Gusev [1], Valeria A. Kostevich [1,4], Nikolay P. Gorbunov [1,4] and Alexey V. Sokolov [1,4]

[1] Department of Biophysics, Federal Research and Clinical Center of Physical-Chemical Medicine of Federal Medical Biological Agency, Moscow 119435, Russia
[2] Department of Medical Biophysics of the Institute for Translative Medicine, Pirogov Russian National Research Medical University, Moscow 117997, Russia
[3] Department of Biophysics, Belarusian State University, 220030 Minsk, Belarus
[4] Department of Molecular Genetics, Institute of Experimental Medicine, St. Petersburg 197376, Russia
* Correspondence: o-panas@mail.ru; Tel.: +7-499-246-44-90

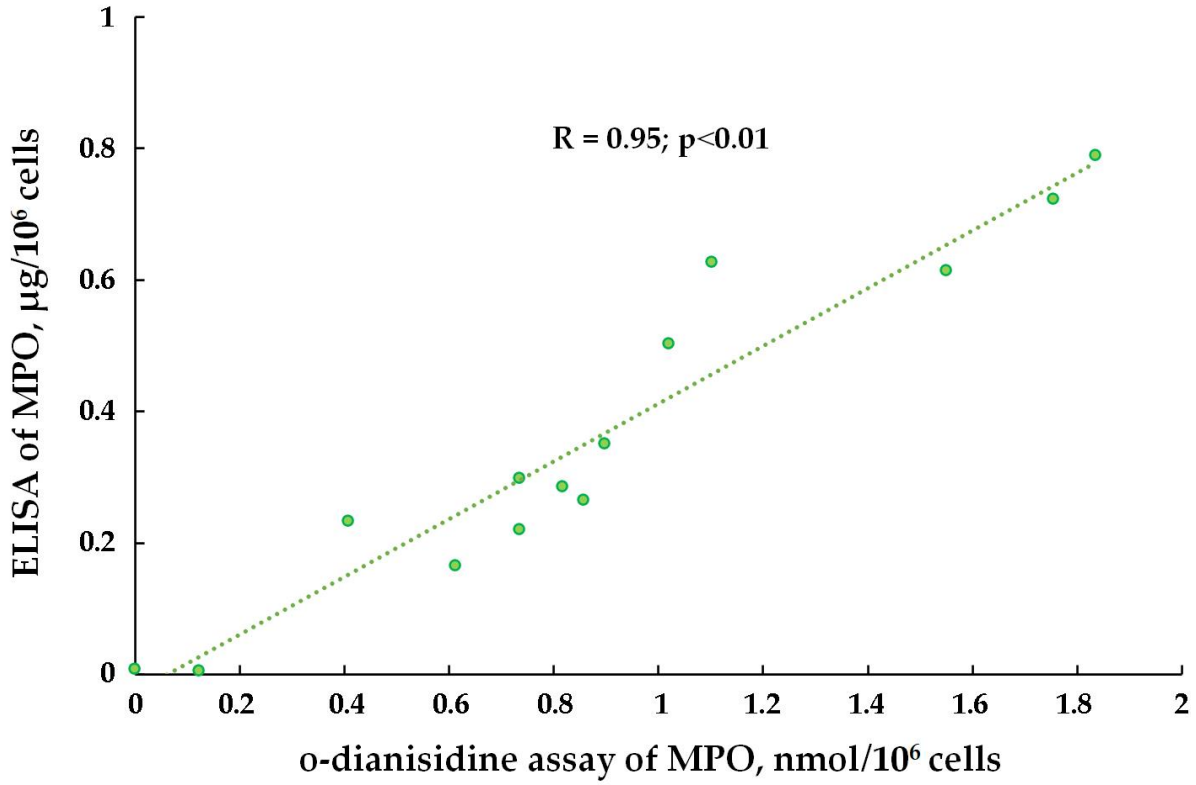

Figure S1. Correlation between MPO peroxidase activity assessed by o-dianisidine test and MPO concentration assayed by ELISA in the supernatants of neutrophils incubated with stimulators in the absence of HSA-MG.

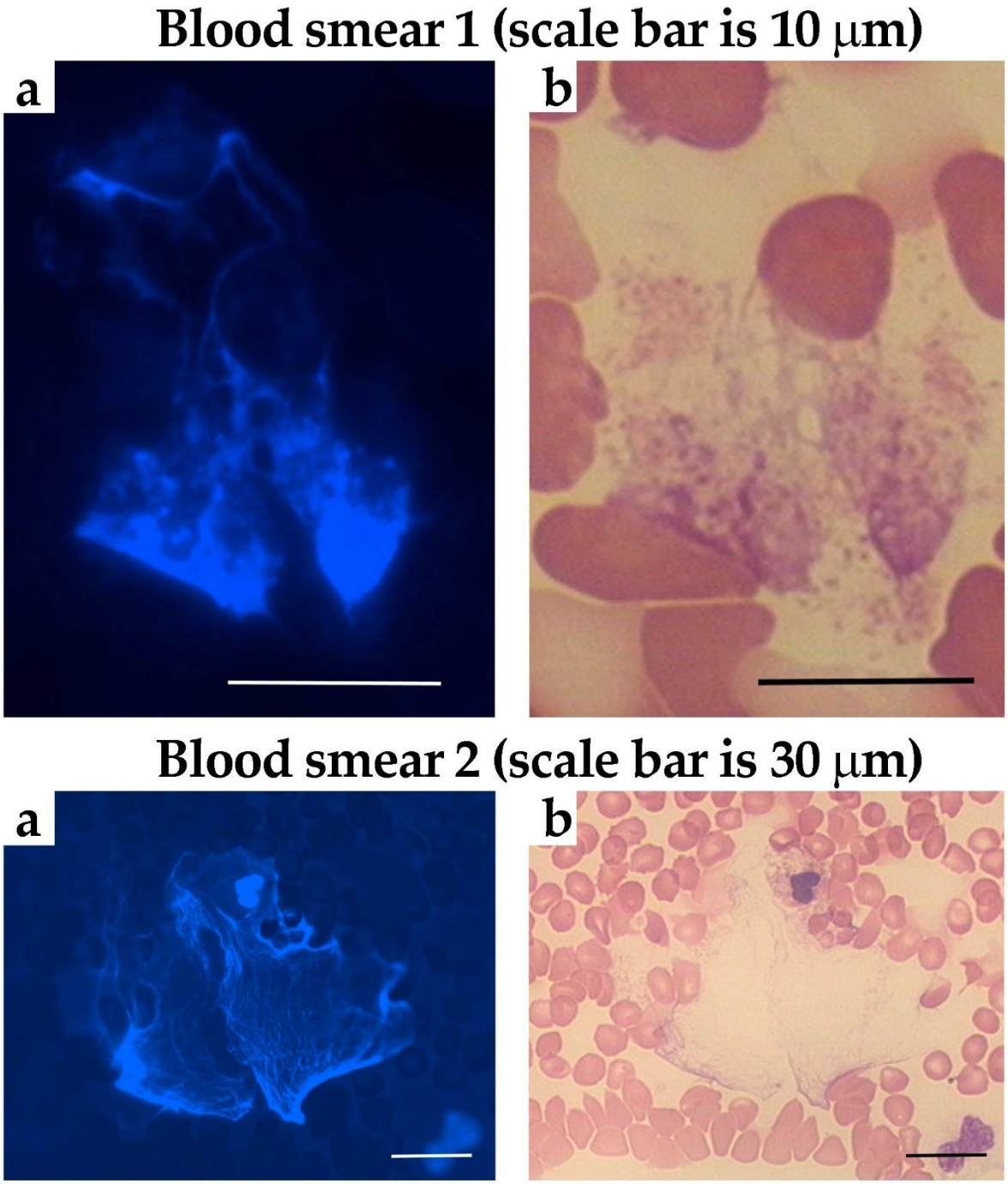

Figure S2. NET-like structures: (a) stained with Hoechst 33342 dye, fluorescent microscopy; (b) stained with Romanowsky dye, light microscopy.

Table S1. Comparison of mAbs against MPO used in study

mAb	Working combination for hemi-MPO detection with HRP-labeled mAb (tested mAb on solid phase)	Detection of dimeric MPO with same HRP-labeled mAb (tested mAb on solid phase)	Resistance of interaction with MPO to 2 M NaCl
1#8	2#7-HRP 4#2-HRP 4#4-HRP 4#8-HRP 4#10-HRP	+	+
2#7	1#8-HRP 4#2-HRP 4#4-HRP 4#8-HRP 4#10-HRP	+	-
4#2	1#8-HRP 2#7-HRP 4#4-HRP 4#8-HRP 4#10-HRP	+	-
4#4	1#8-HRP 2#7-HRP 4#2-HRP 4#4-HRP 4#10-HRP	No detection	-
4#8	1#8-HRP 2#7-HRP 4#2-HRP 4#4-HRP 4#10-HRP	+	-
4#10	No working combinations	No detection	+

Note: For unambiguous characterisation of epitope specificity of solid-phase immobilized and labeled mAbs we used hemi-MPO and not MPO dimer, due to the fact that results obtained with MPO dimer in sandwich ELISA cannot be interpreted in a unique manner (in terms of epitope specificity of mAbs).

Table S2. Comparison of ABTS and CB oxidation in absence and presence of HSA and HSA-MG (n = 3)

Reaction mixture	**ABTS oxidation – A_{414}/min** (1 mM ABTS, 5 nM HRP, 2 mM H_2O_2, 50 mM Na-acetate buffer, pH 5.5)	**CB oxidation – μM HOCl** (200 μM CB, 25 μM HOCl, 150 mM NaCl, 2 mM taurine, 10 μM KI, 20 mM Na-phosphate buffer, pH 5.8)
Control	0.996 ± 0.031	24.8 ± 0.4
500 nM HSA	1.019 ± 0.042	25.1 ± 0.3
500 nM HSA-MG	1.004 ± 0.029	24.9 ± 0.4

Table S3. Comparison of neutrophil functional activity in type 2 diabetes mellitus and the effects of HSA-MG on neutrophils in vitro (see articles [1–9])

Functional activity of neutrophil	Type 2 diabetes mellitus	HSA-MG (this study)
Expression of CD11b	Increased [1–3]	Increased
Expression of CD63, exocytosis of AGs	No effect [4]	No effect
MPO chlorinating activity	Decreased [5,6]	Inhibition
Lum-CL	No effect [5]	No effect
Luc-CL, NADPH-oxidase activity	Increased [6–9]	Increased
NETosis	No effect [4]	No effect

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