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Department of Dermatology and Venereology

Identification of immunometabolic biomarkers and therapeutic targets in granulomatous skin inflammation

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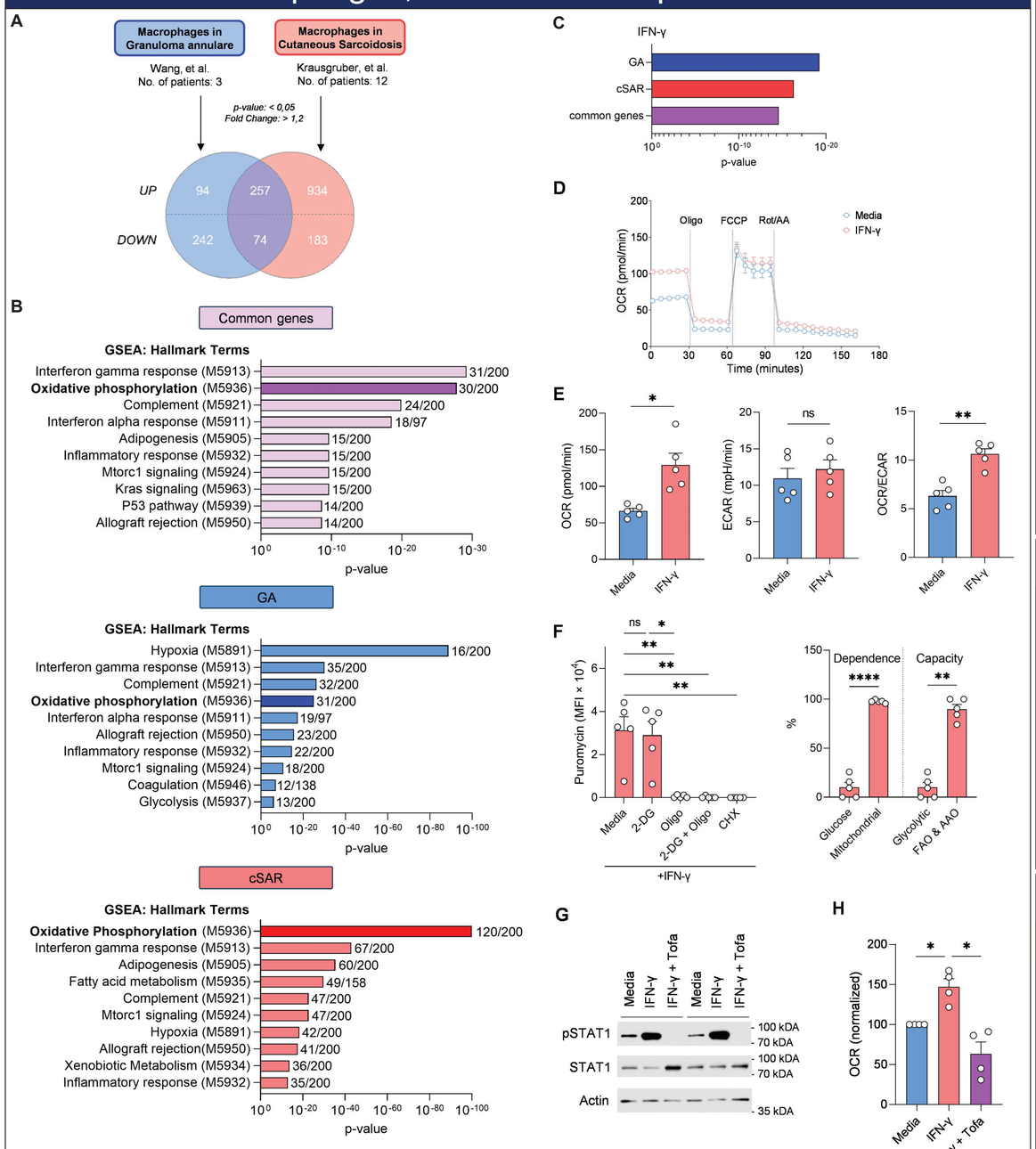
Background and Aims

Granuloma annulare (GA) and cutaneous sarcoidosis (cSAR) are chronic granulomatous skin diseases characterized by macrophage-rich inflammation and may have substantial impact on quality of life.[1,2] IFN-γ–driven macrophage activation is considered central to disease pathogenesis,[3] but the molecular and metabolic mechanisms underlying granuloma formation remain incompletely understood, limiting the development of effective targeted therapies.

Aims:

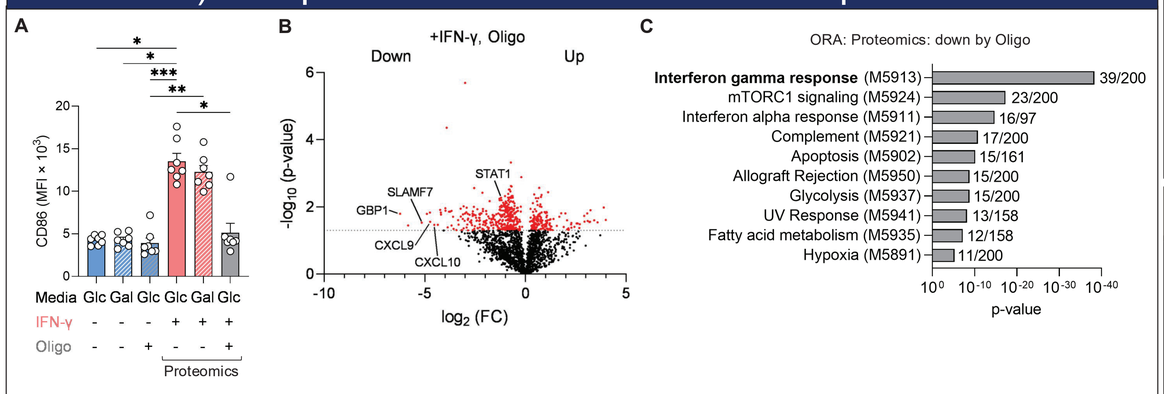
- To identify shared immunometabolic pathways in granuloma annulare and cutaneous sarcoidosis macrophages.
- To assess the role of these metabolic pathways (especially glycolysis, oxidative phosphorylation (ETC), electron transport along the electron transport chain (ETC)) for the granulomatous immune response in macrophages.
- To determine immunometabolic biomarkers and indicators for drug-repurposing strategies applicable to FFPE tissue of GA and cSAR.
- To assess potential macrophage-specific therapeutic strategies in granulomatous inflammation.

1) OXPHOS represents a central metabolic pathway in GA and cSAR macrophages, as well as IFN-γ-activated MDMs.



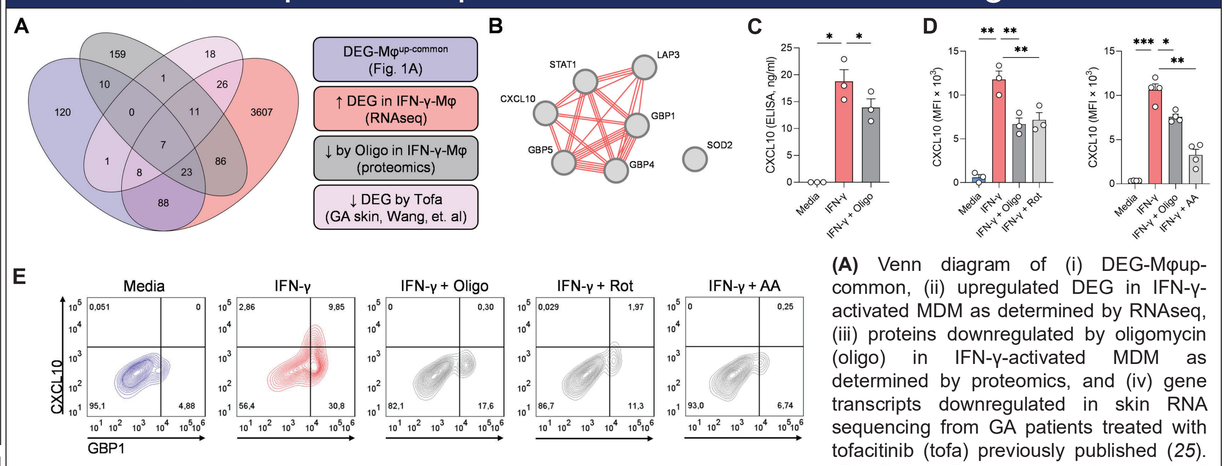
(A) Schematic workflow and venn diagramm for identification of DEG-Mφ$^{all\text{-}GA}$ and DEG-Mφ$^{all\text{-}cSAR}$ from previously published data (Wang et al., 2021; Krausgruber et al., 2023). **(B)** Hallmark term enrichment for DEG-Mφ$^{up\text{-}common}$, DEG-Mφ$^{up\text{-}GA}$, and DEG-Mφ$^{up\text{-}cSAR}$. **(C)** Enrichment of the transcriptional programs associated with IFN-g in DEG-Mφ$^{up\text{-}GA}$, DEG-Mφ$^{up\text{-}cSAR}$ and DEG-Mφ$^{up\text{-}common}$. **(D-F)** MDM were treated with IFN-γ or media (untreated) for 21 hours. **(D)** OCR measured by Seahorse Mito Stress Test. Shown is one representative experiment of five independent experiments with six technical replicates each. **(E)** Basal OCR, ECAR and OCR/ECAR determined by Seahorse (n = 5). **(F)** SCENITH metabolic profile determined by flow cytometry (n = 5). **(G)** Immunoblot of pSTAT1 (Tyr701), STAT1 and Actin in MDM activated with IFN-γ ± Tofacitinib (tofa) or media for 120 minutes. Two representatives out of three donors are depicted (n = 3). **(H)** Basal OCR of MDM treated with IFN-γ ± tofa or media for 21 hours measured by Seahorse (n = 4).

2) IFN-γ activation of MDMs is ETC-dependent.



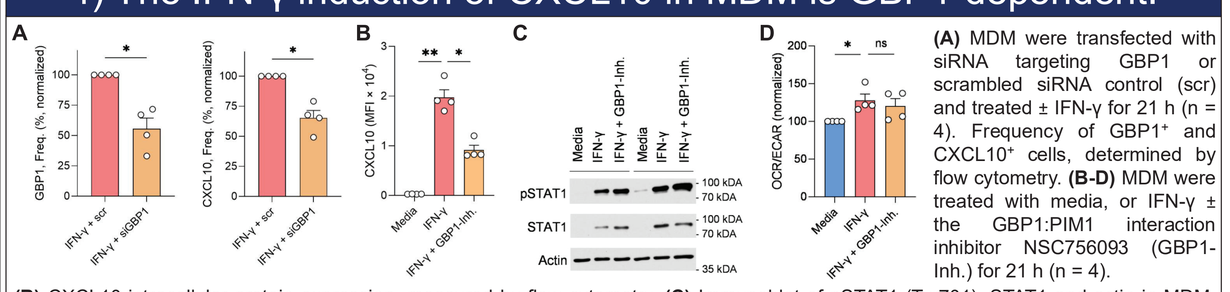
(A-C) MDM were cultured in glucose (Glc)- or galactose (Gal)-containing media, or in Glc-containing media supplemented with oligomycin (oligo), and treated with or without IFN-γ for 21 hours. **(A)** CD86 expression in MDM measured by flow cytometry (n = 7). **(B-D, F)** Proteomics analyses of IFN-γ-activated MDM under the three described conditions (n = 5). **(B)** Volcano plot showing proteome analysis of IFN-γ-activated MDM treated with oligo versus no oligomyin-, Glc-containing media. Red dots indicate significantly regulated proteins. **(C)** Hallmark term enrichment of proteins significantly downregulated by oligo in IFN-γ-activated MDM.

3) GBP1 is a critical regulator and biomarker in an OXPHOS-sensitive, IFN-γ-induced protein network in human skin granulomas.



(A) Venn diagram of (i) DEG-Mφup-common, (ii) upregulated DEG in IFN-γ-activated MDM as determined by RNAseq, (iii) proteins downregulated by oligomycin (oligo) in IFN-γ-activated MDM as determined by proteomics, and (iv) gene transcripts downregulated in skin RNA sequencing from GA patients treated with tofacitinib (tofa) previously published (*25*). **(B)** Protein-Protein interactions of 7 proteins shared in all three data sets of (A) based on STRING analyses. **(C-E)** MDM were treated with media, or IFN-γ ± metabolic inhibitor (oligomycin (oligo), antimycin A (AA) or rotenone (Rot)) for 21 hours. **(C)** CXCL10 in culture supernatants, as determined by ELISA (n = 3). **(D)** CXCL10 intracellular protein expression measured by flow cytometry (n = 3, n = 4, respectively). **(E)** GBP1 and CXCL10 protein expression measured by flow cytometry (n = 4). **(F)** Immunofluorescence microscopy of CD68 (green), GBP1 (red) and DAPI (blue) including merge and respective H.E. in GA and cSAR. Arrow heads: granulomas. Scale bar: 200 µm **(G)** GBP1 MFI of CD68$^+$ cells, based on single cell detection of immunofluorescence staining of normal skin, GA, cSAR (each n = 3). **(H)** Single-cell suspensions from affected skin of GA patients (n = 3), analyzed by flow cytometry. Cells were gated for CD68- and CD68+ events (n = 3). Contour plots show the co-expression of CD40 and GBP1 proteins within these gates. Bar plots depict GBP1 and CD40 expression in CD68$^-$ and CD68$^+$ gated cells.

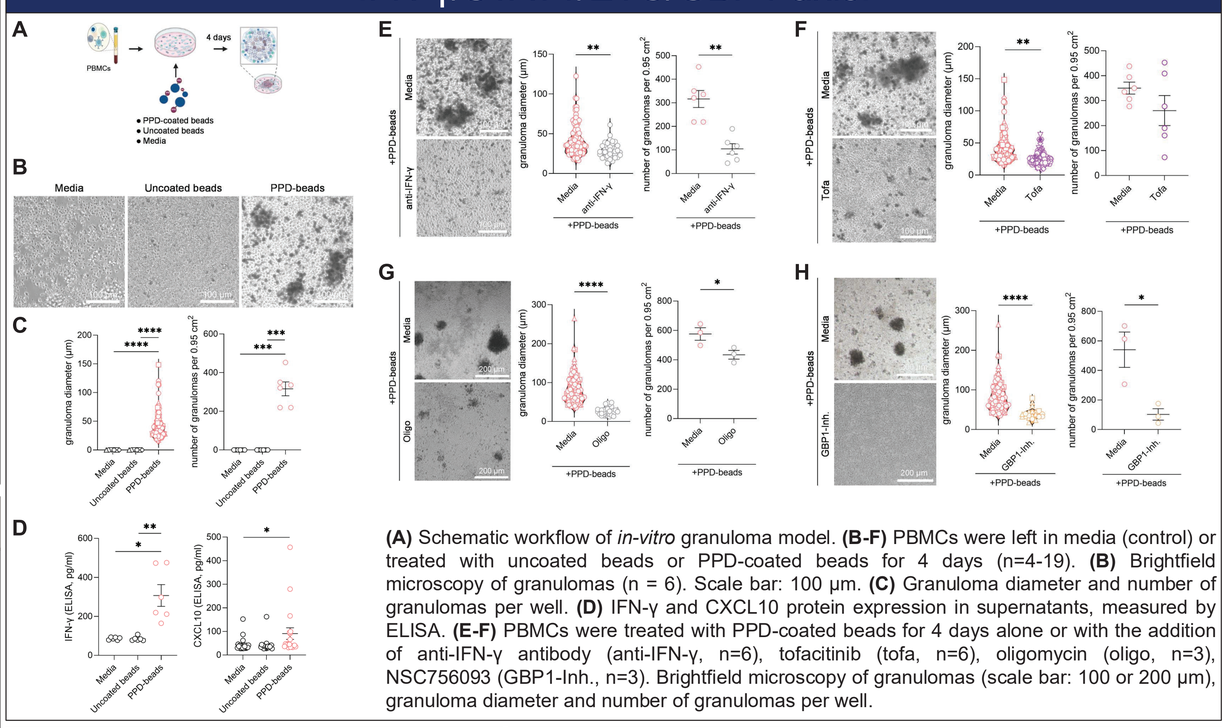
4) The IFN-γ induction of CXCL10 in MDM is GBP1-dependent.



(A) MDM were transfected with siRNA targeting GBP1 or scrambled siRNA control (scr) and treated ± IFN-γ for 21 h (n = 4). Frequency of GBP1$^+$ and CXCL10$^+$ cells, determined by flow cytometry. **(B-D)** MDM were treated with media, or IFN-γ ± the GBP1:PIM1 interaction inhibitor NSC756093 (GBP1-Inh.) for 21 h (n = 4).

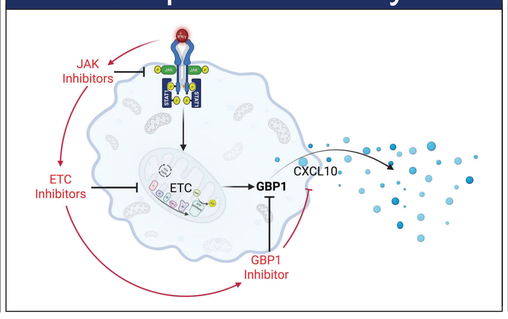
(B) CXCL10 intracellular protein expression, measured by flow cytometry. **(C)** Immunoblot of pSTAT1 (Tyr701), STAT1 and actin in MDM activated with IFN-γ ± GBP1-Inh. or left untreated (media) for 120 min. **(D)** Seahorse OCR/ECAR.

5) *In-vitro* granuloma formation depends on an IFN-γ/STAT1/ETC/GBP1 axis.



(A) Schematic workflow of *in-vitro* granuloma model. **(B-F)** PBMCs were left in media (control) or treated with uncoated beads or PPD-coated beads for 4 days (n=4-19). **(B)** Brightfield microscopy of granulomas (n = 6). Scale bar: 100 µm. **(C)** Granuloma diameter and number of granulomas per well. **(D)** IFN-γ and CXCL10 protein expression in supernatants, measured by ELISA. **(E-F)** PBMCs were treated with PPD-coated beads for 4 days alone or with the addition of anti-IFN-γ antibody (anti-IFN-γ, n=6), tofacitinib (tofa, n=6), oligomycin (oligo, n=3), NSC756093 (GBP1-Inh., n=3). Brightfield microscopy of granulomas (scale bar: 100 or 200 µm), granuloma diameter and number of granulomas per well.

Graphic summary



Outlook

- Defining the precise molecular role of GBP1 in IFN-γ–driven macrophage activation.
- Targeting macrophage metabolism as a promising therapeutic strategy for chronic granulomatous inflammation.
- Validating GBP1 as histological biomarker for therapeutic success of JAK-inhibitors and ETC-inhibitors in GA and cSAR patients.

References:

1) Obi et al. Semin. Respir. Crit. Care Med. 41, 716–732 (2020)
2) Clark et al. Br J Dermatol. 188, 134–136 (2023)
3) Damsky et al., J. Am. Acad. Dermatol. 82, 612–621 (2020).

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