**Supplementary Material**

# LIF/JAK2/STAT1 signaling enhances production of galactose-deficient IgA1 by IgA1-producing cell lines derived from tonsils of patients with IgA nephropathy

Running title: LIF signaling in tonsillar cells, IgA nephropathy

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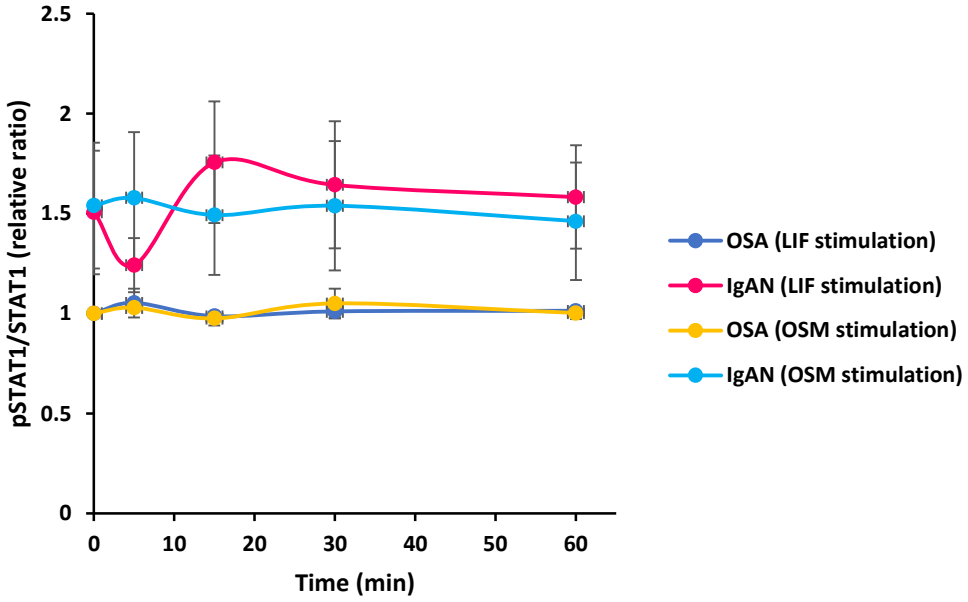
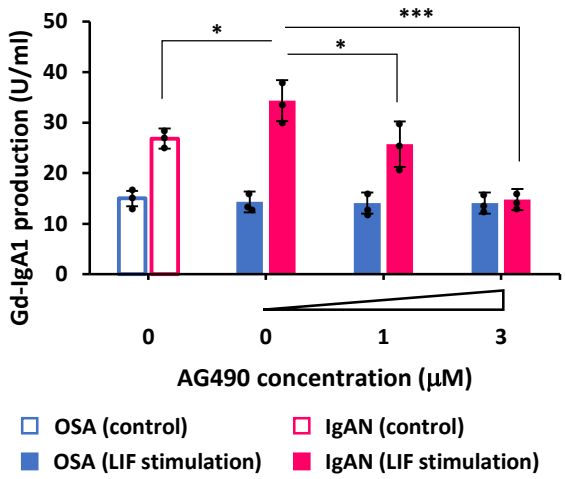
Key words: Genome-wide association studies (GWAS), leukemia inhibitory factor (LIF), oncostatin M (OSM), JAK2 inhibitor, phosphorylated STAT1 (pSTAT1), galactose-deficient IgA1 (Gd-IgA1)



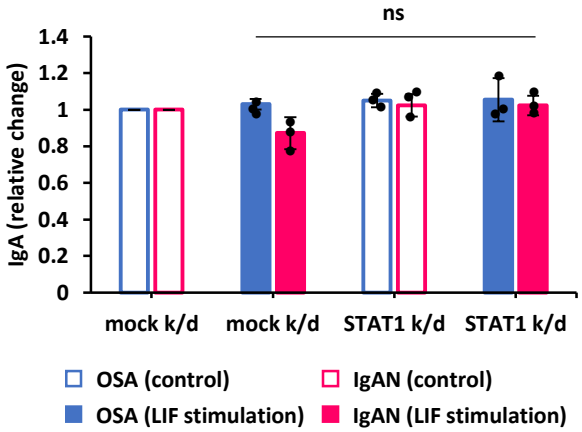

**Supplementary Figure S1.**

**Temporal trends of STAT1 activation on LIF or OSM stimulation.** Analysis of data from experiments shown in Figure 3.



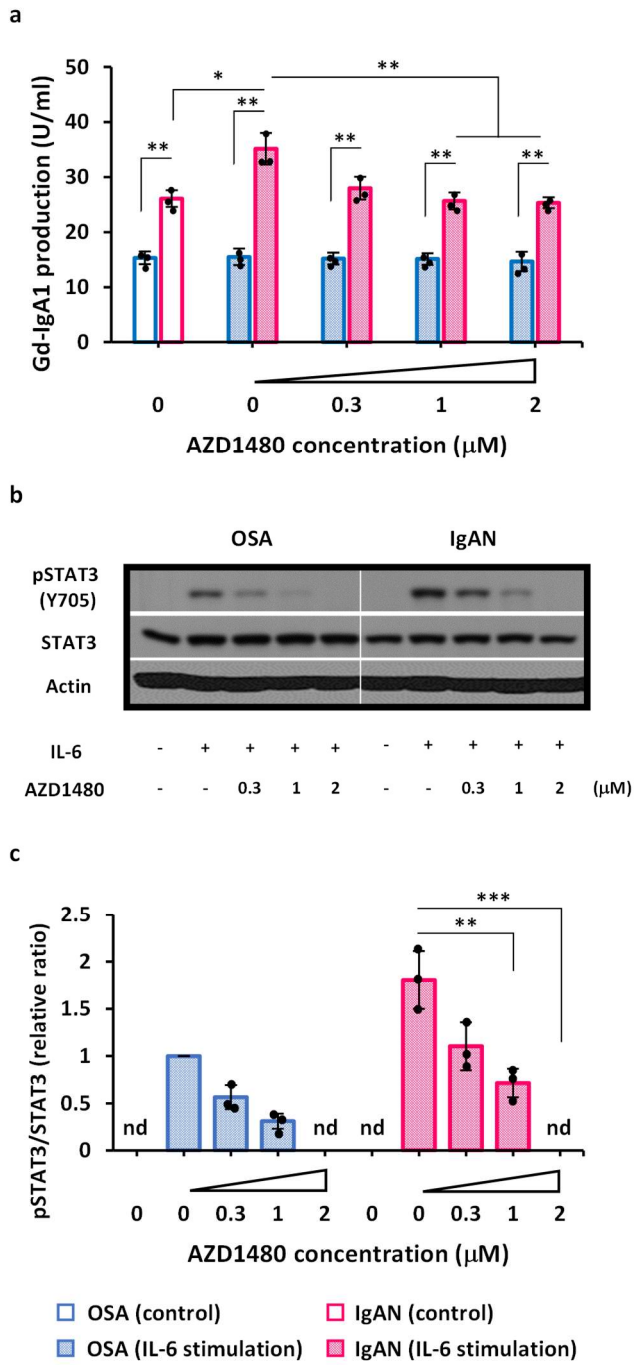

**Supplementary Figure S2.**

**Effect of JAK2 inhibitor AG490 on LIF-mediated Gd-IgA1 overproduction in tonsillar cell lines.** LIF-induced overproduction of Gd-IgA1 by tonsillar IgA1-producing cells from patients with IgAN was inhibited by AG490 in a dose-dependent manner. The cells were pre-incubated with the inhibitor 60 minutes before addition of cytokine. One-hundred Units (100 U) of Gd-IgA1 was defined as 100 ng of the standard Gd-IgA1. Analysis of Gd-IgA1 production was performed for each sample set (n=3 each). Statistical analysis was performed by one-way ANOVA followed by Tukey's multiple comparison test. All data are presented as mean ± SD values and mean values for individual cell lines are shown by black circles. *$P$<0.05, *** $P$<0.005.




**Supplementary Figure S3.**

**IgA production was not altered by STAT1 siRNA knock-down.** Relative change of IgA1 production using siRNA STAT1 knock-down (k/d) with or without LIF stimulation was performed in each sample (n=3). IgA production in mock-control without LIF stimulation in each cell was set to 1. Statistical analysis was performed by one-way ANOVA followed by Tukey's multiple comparison test. All data are presented as mean ± SD values and mean values for individual cell lines are shown by black circles. ns, not statistically significant.




**Supplementary Figure S4.**

**Effect of AZD1480 on IL-6-mediated STAT3 activation and overproduction of Gd-IgA1 by IgA1-secreting tonsillar cell lines.** (a) Gd-IgA1 production in the absence or presence of AZD1480 (0.3-2 μM) in IL-6-stimulated tonsillar IgA-producing cells from OSA (n=3) and IgAN (n=3) patients. The cells were pre-incubated with the inhibitor 60 minutes before addition of cytokine. One-hundred Units (100 U) of Gd-IgA1 was

defined as the OD of 100 ng of the standard Gd-IgA1. (b) Representative images of phosphorylation of STAT3 (pSTAT3) and STAT immunoblots. (c) Densitometric analysis of pSTAT3 of data from B was performed in each sample (n=3). pSTAT3 relative to total STAT3 protein in OSA (IL-6 stimulation without AZD1480) was set to 1. Statistical analysis for (a) and (b) was performed by one-way ANOVA followed by Tukey's multiple comparison test. All data are presented as mean ± SD values and mean values for individual cell lines are shown by black circles. *$P$<0.05, **$P$<0.01, ***$P$<0.005. nd, not detected.

**Supplementary Table S1.**

The primer sets for qPCR of *STAT1* and *GAPDH* genes

| | | |
|---|---|---|
| STAT1 | | |
| | F | 5'-ATGGCAGTCTGGCGGCTGAATT-3' |
| | R | 5'-CCAAACCAGGCTGGCACAATTG-3' |
| GAPDH | | |
| | F | 5'-GTCTCCTCTGACTTCAACAGCG-3' |
| | R | 5'-ACCACCCTGTTGCTGTAGCCAA-3' |

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

| | Item No | Recommendation | Page No |
|---|---|---|---|
| **Title and abstract** | 1 | (*a*) Indicate the study's design with a commonly used term in the title or the abstract | 1, 2-3 |
| | | (*b*) Provide in the abstract an informative and balanced summary of what was done and what was found | 2-3 |
| **Introduction** | | | |
| Background/rationale | 2 | Explain the scientific background and rationale for the investigation being reported | 4-5 |
| Objectives | 3 | State specific objectives, including any prespecified hypotheses | 5 |
| **Methods** | | | |
| Study design | 4 | Present key elements of study design early in the paper | 5, 6-10 |
| Setting | 5 | Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection | 6 |
| Participants | 6 | (*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up | 6 |
| | | (*b*) For matched studies, give matching criteria and number of exposed and unexposed | N/A |
| Variables | 7 | Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable | 6-10 |
| Data sources/ measurement | 8* | For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group | 6-10 |
| Bias | 9 | Describe any efforts to address potential sources of bias | 10 |
| Study size | 10 | Explain how the study size was arrived at | 6 |
| Quantitative variables | 11 | Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings | 6-10 |

| | | | |
|---|---|---|---|
| | | were chosen and why | |
| Statistical methods | 12 | (*a*) Describe all statistical methods, including those used to control for confounding | 10 |
| | | (*b*) Describe any methods used to examine subgroups and interactions | N/A |
| | | (*c*) Explain how missing data were addressed | N/A |
| | | (*d*) If applicable, explain how loss to follow-up was addressed | N/A |
| | | (*e*) Describe any sensitivity analyses | N/A |
| **Results** | | | |
| Participants | 13* | (a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed | N/A (numbe r stated on p 6) |
| | | (b) Give reasons for non-participation at each stage | N/A |
| | | (c) Consider use of a flow diagram | N/A |
| Descriptive data | 14* | (a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders | 6 |
| | | (b) Indicate number of participants with missing data for each variable of interest | 6 |
| | | (c) Summarise follow-up time (eg, average and total amount) | N/A |
| Outcome data | 15* | Report numbers of outcome events or summary measures over time | 11-13, figures, suppl data |
| Main results | 16 | (*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included | N/A |
| | | (*b*) Report category boundaries when continuous variables were categorized | N/A |
| | | (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period | N/A |
| Other analyses | 17 | Report other analyses done—eg analyses of subgroups | N/A |

| | | | |
|---|---|---|---|
| | | and interactions, and sensitivity analyses | |
| **Discussion** | | | |
| Key results | 18 | Summarise key results with reference to study objectives | 14-17 |
| Limitations | 19 | Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias | 15-16 |
| Interpretation | 20 | Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence | 16-17 |
| Generalisability | 21 | Discuss the generalisability (external validity) of the study results | |
| **Other information** | | | |
| Funding | 22 | Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based | 17 |

*Give information separately for exposed and unexposed groups.

**Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.