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TITLE: **Dextran Sulfate, Beta Cell Preservation, and Immune Regulation in Type 1 Diabetes**

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New York, NY 10029

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14. ABSTRACT Type 1 diabetes (T1D), with an incidence that has been increasing by 2-5% worldwide over the past few years, poses a considerable challenge to afflicted individuals, to the development of effective prevention and treatment regimens, and to public health initiatives at large. In T1D, self-tolerance is lost leading to the destruction of insulin-producing cells. Autoreactive T cells acquire an effector inflammatory phenotype due to co-stimulatory signals leading to tissue invasion and insulin-producing cell destruction. Therapies focused on gaining immune tolerance to preserve functional insulin-producing cells are a priority for the treatment of the disease. We found that the semi-synthetic proteoglycan dextran sulfate (DS) decreases diabetes incidence in mice and preserves insulin-producing cells. During the first year of the award, we have determined that DS treatment induces a spectrum of phenotypic changes consistent with a "tolerogenic" modulation of human APC and/or T cell. Alterations in CXCR3, CD62L, CD25, CD38 in T cells and CD123, PDL1 and HLA-DR in APCs are observed and their importance in DS effects will be assessed next year.

15. SUBJECT TERMS
Type 1 Diabetes, Dextran sulfate, human PBMCs, CyTOF, immunomodulation

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TABLE OF CONTENTS

	Page
1. Introduction	7
2. Keywords	7
3. Accomplishments	7
4. Impact	9
5. Changes/Problems	10
6. Products	11
7. Participants & Other Collaborating Organizations	13
8. Special Reporting Requirements	None
9. Appendices	None

DoD progress report Year 1– CyTOF studies

Introduction:
As part of our overarching aim **"to dissect and stratify dextran sulfate (DS)-induced immunomodulatory effects on human T cell responses in health and T1D disease",** we hypothesized that DS exerts tolerogenic effects on human T cell responses by 1., skewing of APC maturation, phenotype and/or function, 2., direct modulation of T cell phenotypes/function, or 3., a combination thereof. In pursuit of this hypothesis, we proposed to employ mass cytometry (MC / CyTOF technology) for a phenotypic and functional stratification of human PBMCs cultured under various experimental conditions according to more than 40 single cell parameters. Specifically, the studies developed during the first year of the award (SA3.1 and 3.2, as indicated in the SOW) were designed as an initial approach to parse the general impact of DS on human T cell and APC populations, and we therefore designed and implemented a 35-parameter exploratory immunophenotyping MC staining panel to characterize the basic phenotypic properties of healthy donor PBMC cultured in the presence/absence of DS and polyclonal T cell stimulation with antibodies directed against CD3 and CD28. Our initial analyses (SA 3.1) demonstrate a principal utility of our proposed experimental approach and featured data documenting that **DS induces specific changes in the monocyte subset consistent with the possibility of reduced antigen-presentation capacity**. To further analyze this significant observation, we have therefore designed and built an APC-focused MC staining panel (SA 3.2) the principal utility of which we have assessed and confirmed APC populations cultured in the presence absence of DS and LPS. Analysis of this second CyTOF experiment is ongoing.

Results and Significance:
In SA3.1, we have interrogated the effects of DS on polyclonal T cell activation (PBMC stimulated with αCD3/αCD28 beads in the absence/presence of DS) using our exploratory MC staining panel and have made the unexpected observation that DS promoted a selective increase of CXCR3 expression by activated $CD8^+$T cells (both in PBMC cultured for 24h and 48h). Since expression of the chemokine receptor CXCR3 is upregulated on effector and memory as compared to naïve $CD8^+$T cells, this observation was at first confounding. However, the kinetics of induced CXCR3 expression by activated CD8+T cells, analyzed in detail by us and others in murine model systems of infectious disease (Hu *et al.*, PMID: 21518913; Eberlein *et al.*, PMID: 27617858), are quite complex and do not permit a simple association between receptor expression levels and activation or developmental $CD8^+$T cell status. Indeed, further stratification of the $CXCR3^+$ $CD8^+$T cell subset in DS cultures revealed that these cells significantly downregulated canonical T cell activation markers such as CD25, CD38 and HLA-DR. Importantly, the CXCR3 receptor and its ligands CXCL9, CXCL10 and CXCL11 have been implicated in both murine and human T1D pathogenesis, and the CXCR3:CXCL9/10/11 axis constitutes a promising target for T1D prevention and/or reversal (reviewed in an editorial we recently authored; van der Heide and Homann, PMID: 27401871). In fact, in our ongoing research independent of the present project we are exploring new ways to target the CXCR3 receptor in a mouse model for virus-induced T1D, and for the purpose of further dissecting the effects of DS on stimulated human $CD8^+$T cells in the present project, we have now designed a T cell-focused MC staining panel that we will deploy in pending experiments for an even more detailed characterization of DS-mediated effects on human $CD8^+$T cell activation (using both unfractioned PBMC cultures as well as FACS-sorted $CD8^+$ and also $CD4^+$T cell populations from non-diabetic donors). The unexpected confluence of DS-dependent CXCR3:CXCL9/10/11 modulation at the level of both T cells and islet cells (RNAseq analysis of human islets treated with cytokines ± DS, studies independent of the current project) constitutes an exciting prospect, and we will leverage our established expertise in this field of T1D research to privilege the pursuit of this particular investigative avenue.
In SA3.2, an investigation into the specific impact of DS on APC maturation, we also adjusted our MC staining panel with a focus on monocytes and myeloid cells and have begun to interrogate the modulation of antigen presenting capacity in LPS-stimulated APC populations as deduced from the expression of HLA-ABC, HLA-DR and a variety of co-stimulatory/inhibitory molecules including PD-L1, PD-L2, CD80, CD86, CD40 and others. Collectively, a phenotype consistent with reduced antigen-presenting function adds to the potentially unique function of DS as an agent for the concurrent tolerogenic modulation of APCs, T cells and beta cells.
In conclusion, our studies developed during the first year are very encouraging and point out to specific immunomodulation by DS that could clearly favor a "tolerogenic" phenotype in autoimmune T1D. This highlights the therapeutic potential of DS for T1D.

1. **INTRODUCTION:** Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

Our studies suggest a promising therapeutic effect of dextran sulfate (DS) for type 1 diabetes (T1D) by potentially inducing immune tolerance. To address this point, we plan to stratify dextran sulfate (DS)-induced immunomodulatory effects on human T cell responses. The hypothesis is that DS exerts tolerogenic effects on human T cell responses by 1) skewing of APC maturation, phenotype and/or function; 2) direct modulation of T cell phenotypes/function; or 3) a combination thereof.

2. **KEYWORDS:** Provide a brief list of keywords (limit to 20 words).

Type 1 diabetes, dextran sulfate, immune tolerance, T cell, antigen presenting cell, mass cytometry / MC, CyTOF, human PBMCs.

3. **ACCOMPLISHMENTS:** The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.

What were the major goals of the project?
List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

The goals for the first year of this project are:
SA3.1: To parse the general impact of DS on human T cell and APC populations (6 months, Homann and Garcia-Ocana PIs). For these experiments we have cultured unfractioned fresh human PBMCs from healthy donors with dextran sulfate (DS) for 12-48h and performed initial phenotypic characterization of T cell and APC populations by Cytometry by Time of Flight (CyTOF). These experiments were performed in the absence or presence of anti-CD3/anti-CD28 stimulation to specifically evaluate the impact of DS in the context of T cell activation.
SA3.2: To delineate the specific impact of DS on APC maturation (50% of 8 months, Homann and Garcia-Ocana PIs). For these experiments we have cultured unfractioned human PBMCs from healthy donors with DS for 24h in the absence or presence of LPS and are performing initial phenotypic characterization of APC populations by CyTOF.

What was accomplished under these goals?
For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

In SA3.1, we interrogated the effects of dextran sulfate (DS) on T cell activation (PBMC stimulated with αCD3/αCD28 beads in the absence/presence of DS) using our exploratory MC staining panel and have made the unexpected observation that DS promoted a selective increase of CXCR3 expression by activated CD8+T cells. Since expression of the chemokine receptor CXCR3 is upregulated on effector and memory as compared to naïve CD8+T cells, this observation was at first confounding. However, further stratification of the CXCR3+ CD8+T cell subset in DS cultures revealed that these cells significantly downregulated canonical T cell activation markers such as CD25, CD38 and HLA-DR. Importantly, the CXCR3 receptor and ligands CXCL9/10/11 have been implicated in T1D pathogenesis, and the CXCR3:CXCL9/10/11 axis constitutes a promising target for T1D prevention and/or reversal of the disease. Next, we have designed a T cell-focused MC staining panel for an even more detailed characterization of DS-mediated effects on human CD8+T cell activation. In SA3.2, we have begun to interrogate DS modulation of antigen presenting capacity in LPS-stimulated APCs as deduced from expression of HLA-ABC, HLA-DR, PD-L1/L2, CD80, CD86, CD40 and others. Collectively, a phenotype consistent with reduced antigen-presenting function adds to the potentially unique function of DS as an agent for the concurrent tolerogenic modulation of APCs, T cells and beta cells.

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Training on CyTOF data analysis was provided by:
- Dr. Adeeb Rahman, PhD, Assistant Professor, Genetics and Genomic Sciences, Director of Technology, Human Immune Monitoring Center at Icahn School of Medicine at Mount Sinai.
- Dr. Amir Horowitz, PhD, Assistant Professor, Oncological Sciences, Precision Immunology Institute at Icahn School of Medicine at Mount Sinai.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Nothing to report.

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

Next year we will finish the experiments in SA 3.2 as indicated in the SOW and delineate the specific impact of DS on APC maturation. In addition, we will start SA 3.3. and define the impact of DS preconditioning on islet antigen-reactive CD4+T cell responses. For this, we have already established a collaboration with Dr. Sally Kent at U. Mass who is an expert in human T cell clone generation and will train us on generating these clones for the studies proposed in SA 3.3. Immature DCs will be pre-conditioned with DS during maturation and during the last 4h of maturation, DCs will be incubated with synthetic peptides, after which DCs will be cultured with CFSE-labeled antigen-reactive CD4 T cell clones. Proliferation and cytokine production by the CD4 clones will be determined.

4. **IMPACT:** Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

The unexpected confluence of dextran sulfate-dependent chemokine expression modulation at the level of both T cells and islet cells together with a phenotype consistent with reduced antigen-presenting function adds to the potentially unique function of dextran sulfate as an agent for the concurrent tolerogenic modulation of APCs, T cells and beta cells. In conclusion, our studies developed during the first year point out to specific immunomodulation by dextran sulfate treatment that could clearly favor a "tolerogenic" phenotype in autoimmune type 1 diabetes. This highlights the therapeutic potential of dextran sulfate for type 1 diabetes.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

The results obtained thus far indicate an immunomodulatory effect of dextran sulfate in antigen presenting cells and T cells highlighting the therapeutic potential of this agent for autoimmune type 1 diabetes. Therefore, dextran sulfate can have potential applicability for other autoimmune diseases such as lupus, rheumatoid arthritis, thyroiditis, etc. Importantly, our current studies can have an important impact in other medical disciplines.

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to report.

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to report.

5. **CHANGES/PROBLEMS:** The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

No changes in scope, objectives or approach.

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

No anticipated problems or delays during the project.

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

No significant impact on expenditures.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

No changes in use of human subject samples.

Significant changes in use or care of vertebrate animals

No animals are involved in this project.

Significant changes in use of biohazards and/or select agents

No changes in biohazards and/or select agents.

6. **PRODUCTS:** List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

No Journal publications for this first year of the award.

  Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

No books or other non-periodical, one time-publications for this first year of the award.

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Oral presentation: "Immunomodulatory and beta cell cytoprotective actions of dextran sulfate in T1D". Zihan Zheng, Geming Lu, Juan Carlos Alvarez-Perez, Dirk Homann and Adolfo Garcia-Ocana. 26th Annual Boston Ithaca Islet Club Meeting. April 28-29, 2018. UMass Medical School, Worcester, MA

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

No websites or internet sites.

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

No new technologies or techniques.

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

No inventions, patent applications, and/or licenses.

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

- *data or databases;*
- *physical collections;*
- *audio or video products;*
- *software;*
- *models;*
- *educational aids or curricula;*
- *instruments or equipment;*
- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*
- *other.*

No other products.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?
Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Name: Dirk Homann; Project Role: PI; Researcher Identifier (e.g. ORCID ID): orcid.org/0000-0002-7622-5754
Nearest person month worked: 11
Contribution to Project: Directing, reviewing and interpreting the results of the project.
Funding Support: No additional funding for this project.

Name: Adolfo Garcia-Ocana; Project Role: PI; Researcher Identifier (e.g. ORCID ID): orcid.org/0000-0002-6883-6176
Nearest person month worked: 11
Contribution to Project: Directing, reviewing and interpreting the results of the project.
Funding Support: No additional funding for this project.

Name: Zihan Zhang; Project Role: Co-investigator; Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 11
Contribution to Project: hPMBCs treatments, Flow cytometry and analysis of CyTOF data.
Funding Support: No additional funding for this project.

Name: Jessica Wilson; Project Role: Co-investigator; Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 11
Contribution to Project: Cell culture and hPMBCs treatments.
Funding Support: No additional funding for this project.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Previous Grant Support that Ended:

Helmsley Charitable Trust 2015PG-T1D052 "The nPOD Autoimmunity Group";
ended on 10/31/17

NIH P30DK02054141
Einstein-Mount Sinai DRC Pilot Grant FS 483 "Inclusive interrogation of complex native human islet cell populations by MC"
ended on 03/31/18

NIH/NIAID R21AI126009
"Decay accelerating factor-dependent inhibition of T cell immunity"
officially ended on 05/31/18 but currently in NCE

Pending Grants that are now Active:

NIH R21ES027916
Title: "Mapping Essential Elements and Toxic Metals in the Human Pancreas in Health and Disease"
Role: MPI (Homann & Arora)
Amount: $150,000 ADC
Funding period: 12/15/2017 – 11/30/2019

NIH R01AI134971
Title: "Integrated functional histopathology of the diabetic human pancreas"
Role: MPI (Homann & von Herrath); 2.4 calendar months
Amount: $400,000 ADC
Funding Period: 12/07/2017 – 11/30/2022

NIH UC4DK116284
Title: "Human islet infiltrating T cell biology: reactivity, structure, and function"
Role: Co-Investigator (Kent, Harlan & Stern, PIs)
Amount: $84,920 (total portion)
Funding period: 09/15/2017 – 08/30/2021

What other organizations were involved as partners?

If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: None

QUAD CHARTS: None

9. APPENDICES: None