CBC | CHICAGO BIOMEDICAL CONSORTIUM

CBC Catalyst Award Proposal Title Page

Title of the proposal *(no more than 100 characters including white space)*

NOVEL STRATEGIES TO DRIVE ANTIGEN SPECIFIC TOLERANCE AGAINST RESPIRATORY ALLERGENS IN ASTHMA

Name, degree, title, institution, and contact information including the email address of each PI

Stephanie C. Eisenbarth, M.D. Ph.D.
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Do you have any current or pending grant applications that potentially overlap with this application?
Check ONE:

YES ☐ **NO** ☒

If YES, please identify them.

Not applicable

Explain the overlap in the Biosketch section.

Does the proposed research involve animal subjects?	**Check ONE:**	**YES** ☒	**NO** ☐
Does the proposed research involve human subjects?	**Check ONE:**	**YES** ☐	**NO** ☒
Does the proposed research involve embryonic stem cells?	**Check ONE:**	**YES** ☐	**NO** ☒

(Note: if the data entered exceeds one page, it is acceptable to submit it as two pages.)

Lay-summary: Asthma affects more than 300 million people worldwide, including more than 25 million people in the U.S. alone, and its prevalence has increased over the last few decades.[1,2,3] Allergic asthma is the most common type of asthma, for which there is no cure. Current treatments manage the disease symptoms by broadly suppressing the immune system, which leads to detrimental side effects and respiratory infections.[4] We will develop novel biomaterials that harness the body's intrinsic tolerance inducing mechanisms to reverse allergy and treat asthma without compromising respiratory immunity. Specifically, we will educate the immune system to ignore respiratory allergens by modifying them through protein engineering and synthetic chemistries. We will employ advanced flow cytometry to robustly characterize and gain better understanding of the specific cell types that suppress the immune response to respiratory allergens.

Significance and Innovation: Aberrant immune activation against innocuous allergens remains a central cause of allergic airway disease.[5] Conventional treatments focus on symptoms rather than the underlying immune response driving the disease[3,6]; additionally, they cause detrimental side effects and broad immune suppression.[6] In contrast, **our approach** is to target the body's endogenous pathways of peripheral immune tolerance, training the immune system to be unresponsive to innocuous allergens, thus addressing the central cause of allergic airway disease. Our therapeutic platform breaks the current paradigm of broad immunosuppression that would increase patient susceptibility to infection, and thus is a novel and innovative approach with a great potential towards a cure for asthma. **We propose** to perform protein engineering and synthetic chemistries to actively direct respiratory allergens towards efferocytic pathways, which mediate the clearance of dead cells and prevent the body from mounting an immune response against itself. **Our goal** for this Catalyst proposal is to demonstrate how we can harness the body's endogenous tolerance-inducing mechanisms to address the central cause of asthma and encourage the development of new therapeutics that focus on inducing tolerogenic responses to treat allergic disease.

Proposal statement

Description of proposed research: Efferocytosis, the non-immunogenic clearance of dying cells by antigen presenting cells, is a pathway that induces endogenous peripheral immune tolerance.[7] The liver plays fundamental physiological roles in developing and maintaining tolerance, as efferocytosis is performed by multiple hepatic antigen presenting cells (hAPCs) such as hepatocytes, liver sinusoidal endothelial cells (LSECs), Kupffer cells (KCs), and dendritic cells (DCs).[8,9] Antigens taken up by hAPCs through efferocytic pathways are presented in a tolerogenic manner, and thus drive immune tolerance through T cell deletion, anergy, and regulatory T cell (Treg) conversion.[10,11] Here, we propose a two-pronged approach in which we actively target allergens to hAPCs to achieve antigen-specific tolerance. On one hand, through protein engineering, we will develop Gas6-allergen fusion proteins that will target the efferocytic receptors Axl and Mertk, which are specifically present in KCs.[12,13] On the other hand, through synthetic chemistries, we will develop mannosylated allergens that target the mannose receptor present in multiple hAPCs that mediate efferocytosis, such as LSECs, KCs, and DCs.[10,14] **Our goals for this Catalyst Award are to (Aim 1) engineer respiratory allergens, targeting them to efferocytic pathways to develop antigen-specific tolerance, and (Aim 2) to test whether these biomaterials can specifically suppress aberrant immune responses against respiratory allergens and thus ameliorate allergic airway disease.**

Aim 1: Design and develop engineered respiratory allergens to target the efferocytic liver microenvironment

Rationale: Efferocytic cells in the liver can be actively targeted through engagement of their surface receptors. The first step is to develop variants of the model respiratory allergen, chicken ovalbumin (OVA), and the clinically relevant, immunodominant allergen, Der p 1 present in house dust mite extract (HDM). These modifications include protein engineering to develop the fusion proteins $Gas6_{OVA}$ and $Gas6_{Derp1}$, and synthetic protein mannosylation to develop $pMan_{OVA}$ and $pMan_{Derp1}$, to harness efferocytic pathways and test the development of antigen-specific tolerance.

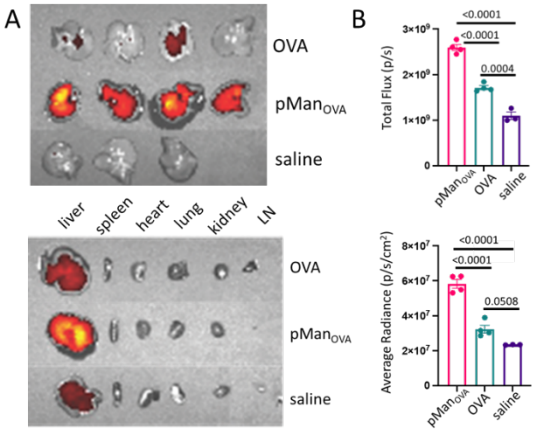

Figure 1: Experimental strategy for biomaterial liver targeting. A. Fluorescent antigen was injected intravenously into mice, and whole organs were imaged by IVIS. B. IVIS quantification show enhanced liver-targeting using pMan$_{OVA}$.

Experimental Methods: To confirm that the Gas6-allergen fusion proteins can target the liver microenvironment, we will perform biodistribution studies after intravenous injection of our fluorescently labelled fusion proteins. We will use an in vivo imaging system (IVIS) to image and determine protein distribution across whole organs, and complement this with flow cytometry and immunofluorescence (IF) microscopy to assess the specific cell types that take up our biomaterials. To determine whether OVA is presented by hAPCs, a critical step for tolerance induction, we will infuse mice with transgenic OT-II T cells, which recognize and respond to a peptide derived from OVA when it is presented by hAPCs.[15]

Anticipated results: We expect that our engineered molecular modifications will target allergens to the liver microenvironment, where presentation by hAPCs will drive tolerant responses. IVIS imaging will demonstrate antigen localization to the liver, and through flow cytometry and IF microscopy we will determine the distribution of antigen among hAPC subtypes. Finally, we expect that hAPC presentation will drive tolerant responses, characterized by deletion, anergy, or Treg conversion of infused OT-II cells.

Alternative strategies: If Gas6-allergen fusion proteins do not effectively target the liver microenvironment, we will rely on synthetic mannosylation of allergens, as the Hubbell lab has previously validated liver targeting with this platform (Fig 1). This molecular engineering can be performed on both OVA and Der p 1.

Aim 2: Determine if active targeting to efferocytic cells in the liver drives antigen-specific tolerance to respiratory allergens and ameliorates allergic airway inflammation.

Rationale: It is unknown whether antigen-specific tolerance can be achieved against respiratory allergens by targeting the efferocytic pathways in the liver microenvironment. In collaboration with the Eisenbarth laboratory, we will test the therapeutic efficacy of the Gas6-allergen fusion proteins and the pMan-conjugated allergens at ameliorating disease in mouse models of allergic airway inflammation.

Experimental Methods: We will first implement an OVA model of allergy. Mice are systemically sensitized to OVA, then challenged through intratracheal exposure to OVA to recapitulate pathological presentations of allergic airway disease. We will use bronchoalveolar lavage (BAL) to evaluate airway eosinophilia, a key clinical readout in BAL fluid samples from asthmatics. We can assess additional pathological readouts, including quantification of serum IgG1 and IgE levels by ELISA, and histological identification of mucus hyper secretion in the lungs by Periodic acid Schiff staining. We will test the effectiveness of Gas6$_{OVA}$ and pMan$_{OVA}$ in preventing airway disease when introduced in both a prophylactic and a therapeutic manner. Furthermore, we will capitalize on the well characterized and widely used transgenic OT-II T cells to study OVA-specific immune responses, as these will allow us to better characterize and understand the mechanisms by which antigen-specific tolerance is achieved. These experiments will inform our subsequent studies utilizing HDM, a clinically relevant human allergen. In the HDM model of allergy, mice are sensitized and challenged through intratracheal HDM exposure only, better mimicking asthmatic sensitization when compared to the OVA model. We will assess the effectiveness of Gas6$_{Derp1}$ and pMan6$_{Derp1}$ in ameliorating the same pathological presentations of allergic airway disease described above, as improvements in this model would imply a benefit at disease management in human patients. Altogether, these studies will provide the basis for a novel therapeutic platform that treats the root cause of the aberrant immune response to innocuous allergens present in asthmatic patients.

Anticipated results: Treatment with our biomaterials will reduce lung and airway eosinophilia, ameliorate mucus production, and lower the levels of allergic antibodies in the sera. Furthermore, we anticipate that treatment with our engineered allergens will drive OT-II T cells into a Treg phenotype, thus suppressing allergic airway disease.

Alternative strategies: Using the mechanistic (OVA) model of airway inflammation, we can utilize the OT-II transgenic T cells to better understand the tolerance-inducing mechanisms of our biomaterials. Because the HDM is an allergen mixture (similar to what human asthmatics are exposed and respond to), there are no available transgenic T cells to study HDM-specific immune responses. To address this limitation, we will use Der p 1-specific tetramers to identify allergen-specific T cells responding to the immunodominant protein in the HDM and characterize their functions.

Nature of inter-institutional collaboration: The proposed project involves the development, characterization, and engineering of novel biomaterials and their application in a complex immunological and pathological system. Thus, our collaboration between the Hubbell laboratory and the Eisenbarth laboratory is crucial for the success of this project. Jeffery Hubbell, Ph.D. (University of Chicago) is a senior investigator and a leader in molecular engineering, which focuses on the development of synthetic and recombinant biological materials. The Hubbell laboratory has performed extensive work on developing novel therapeutic biomaterials to enhance drug delivery for the treatment of autoimmune diseases[16,17] and cancer[18,19], and thus has state of the art infrastructure to produce, isolate, characterize, and modify materials that can modulate allergic immune responses associated to asthma. Stephanie Eisenbarth, Ph.D., M.D. (Northwestern University) is a young investigator and clinician scientist, and a newly appointed professor in the Department of Medicine, Division of Allergy and Immunology. The Eisenbarth lab has focused on dissecting and understanding the pathogenesis of allergic airway inflammation[20] associated with asthma, as well as the pathogenesis of other allergic disorders such as atopic dermatitis and food allergies.[21,22] They pioneered and improved mouse models of allergic airway disease[23], characterizing both the pathological hallmarks of the disease as well as the immunological features that are causative of the allergic response. Therefore, the proposed collaboration capitalizes on the expertise of both the Hubbell and Eisenbarth labs, and their contributions are essential to the success of this research. The two PI's will hold bi-weekly conference calls, monthly in-person meetings (alternating at each institution) and will jointly attend allergy conferences to ensure the project's success.

Criteria for measuring the success of the proposed project: Our proposed project can be divided into two main arms: the development and characterization of modified allergens that actively target efferocytic receptors (Aim 1), and the dosing and testing of their therapeutic efficacy at ameliorating allergic airway disease (Aim 2). In the Hubbell lab, we have successfully developed $Gas6_{OVA}$ and $pMan_{OVA}$ engineered allergens. We propose to perform similar modifications to Der p 1, a clinically relevant allergen which 60% of all asthmatics respond to. Our success criteria for Aim 1 will be the development of the glycosylated and recombinant allergens, and successful demonstration of active targeting to efferocytic cells in the liver. To evaluate the effectiveness of our biomaterials, we will assess their therapeutic efficacy using both mechanistic (OVA) and clinically relevant (HDM) models of allergic airway inflammation. With the expertise of the Eisenbarth lab, we will test the abilities of these materials at ameliorating the pathological presentations of allergic airway disease, including lung and airway eosinophilia, increased systemic levels of IgG1 and IgE antibody isotypes, and mucus hypersecretion. Furthermore, we will use the OVA model of allergy to better understand the mechanisms by which our biomaterials induce antigen-specific tolerance. Given the limited tools available to study HDM-mediated immune responses, our success criteria will be to demonstrate the ability of our Der p 1 therapeutics to ameliorate allergic airway disease. Improvements of disease outcomes at this stage of the project would suggest a clinical benefit when treating asthmatic patients.

Long-term funding plan for continuation beyond the Catalyst Award:
The Hubbell lab is funded to study tolerance-inducing biomaterials, specifically in the context of multiple sclerosis (MS), and antibody responses against enzyme therapeutics. This provides a foundation upon which to study these novel biomaterials in other disease contexts such as allergic airway inflammatory responses associated with asthma. Neither laboratory has funding to study efferocyte-targeting biomaterials in the context of allergic airway disease. Our goal is to use the molecular tools and data generated in this study to apply for NIH funding (R01 or P01) at the conclusion of the first year of the Catalyst Award.

References:

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Program Director/Principal Investigator (Last, First, Middle): Hubbell, Jeffrey

DETAILED BUDGET FOR INITIAL BUDGET PERIOD
DIRECT COSTS ONLY

FROM 06/01/2023 THROUGH 05/31/2025

List PERSONNEL *(Applicant organization only)*
Use Cal, Acad, or Summer to Enter Months Devoted to Project
Enter Dollar Amounts Requested *(omit cents)* for Salary Requested and Fringe Benefits

NAME	ROLE ON PROJECT	Cal. Mnths	Acad. Mnths	Summer Mnths	INST.BASE SALARY	SALARY REQUESTED	FRINGE BENEFITS	TOTAL
Jeffrey Hubbell	PD/PI	0	0	0	213,417	0	0	0
Jorge E. Gomez Medellin	Postdoc	12	0	0	55,000	55,000	13,915	68,915
								0
								0
								0
								0
								0
SUBTOTALS →						55,000	13,915	68,915

CONSULTANT COSTS

EQUIPMENT *(Itemize)*

SUPPLIES *(Itemize by category)*
Cost of allergen proteins: $2,318; Lung and allergy specific antibodies and legendplex: $1,000; Fluorescently labeling products: $600; Production of allergen plasmids: $500

4,418

TRAVEL

INPATIENT CARE COSTS

OUTPATIENT CARE COSTS

ALTERATIONS AND RENOVATIONS *(Itemize by category)*

OTHER EXPENSES *(Itemize by category)*
Animal purchase $5,000; per diem animal costs: $5,000

10,000

CONSORTIUM/CONTRACTUAL COSTS | DIRECT COSTS

SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD *(Item 7a, Face Page)* $ 83,333

CONSORTIUM/CONTRACTUAL COSTS | FACILITIES AND ADMINISTRATIVE COSTS

TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD $ 83,333

Program Director/Principal Investigator (Last, First, Middle): Hubbell, Jeffrey

BUDGET FOR ENTIRE PROPOSED PROJECT PERIOD
DIRECT COSTS ONLY

BUDGET CATEGORY TOTALS	INITIAL BUDGET PERIOD *(from Form Page 4)*	2nd ADDITIONAL YEAR OF SUPPORT REQUESTED	3rd ADDITIONAL YEAR OF SUPPORT REQUESTED	4th ADDITIONAL YEAR OF SUPPORT REQUESTED	5th ADDITIONAL YEAR OF SUPPORT REQUESTED
PERSONNEL: *Salary and fringe benefits. Applicant organization only.*	68,915	70,982			
CONSULTANT COSTS					
EQUIPMENT					
SUPPLIES	4,418	2,351			
TRAVEL					
INPATIENT CARE COSTS					
OUTPATIENT CARE COSTS					
ALTERATIONS AND RENOVATIONS					
OTHER EXPENSES	10,000	10,000			
DIRECT CONSORTIUM/ CONTRACTUAL COSTS					
SUBTOTAL DIRECT COSTS *(Sum = Item 8a, Face Page)*	83,333	83,333	0	0	0
F&A CONSORTIUM/ CONTRACTUAL COSTS					
TOTAL DIRECT COSTS	83,333	83,333	0	0	0

TOTAL DIRECT COSTS FOR ENTIRE PROPOSED PROJECT PERIOD	**$** 166,666

JUSTIFICATION. Follow the budget justification instructions exactly. Use continuation pages as needed.

See attached continuation page below.

Budget Justification

A. PERSONNEL

Jeffrey Hubbell, Ph.D., Principal Investigator

Dr. Hubbell will be responsible for coordinating and supervising the Hubbell laboratory's portion of the grant, focused on the engineered allergens. This includes hiring, training, and supervising staff and students, providing expertise and experience, and data analysis. In addition, Dr. Hubbell will conduct the coordination meetings between the Eisenbarth and Hubbell laboratories, and will be responsible, along with Dr. Eisenbarth, for reporting the study's findings.

B. OTHER PERSONNEL

Jorge E. Gomez Medellin, Ph.D., Post-Doctoral associate (effort = 12 academic and calendar months in the 1st year and 2nd year). Dr. Gomez Medellin will be responsible for designing innocuous allergen proteins and producing and purifying these in sufficient quantities to dose and tolerize mice through the intravenous route. Dr. Gomez Medellin will also be responsible for dosing and tolerizing mice in mouse models of allergic airway disease and will assist in histology and flow cytometric analysis. Dr. Gomez Medellin will perform data analysis and manuscript preparation. Additionally, Dr. Gomez Medellin will be responsible for ordering supplies and planning the Hubbell laboratory day-to-day management of this study.

C. SUPPLIES

Allergen proteins: We will need recombinant Der p 1, an immunodominant protein in the house dust mite allergen extract to synthetically glycosylated and deliver it to the liver microenvironment.

Production of allergen plasmids: To perform protein engineering and generate the recombinant Gas6-Der p 1, we require the production of allergen plasmids for expression in mammalian cell lines. We estimate costs for the design and production of the plasmids up to $500.

Fluorescently labelling products: To perform biodistribution studies of our engineered allergens, we will need to fluorescently label these and track them across and within organs. We estimate a total of $600 for fluorescent labelling kits to perform such experiments.

Antibody products (lung and allergy specific, and legendplex): To analyze allergic inflammation in mouse models, we will require antibodies to identify specific cell populations that mediate lung allergy. Similarly, to assess the extent of the allergic response, we will perform in-vitro restimulation assays of lung immune cell populations. To assess multiple effector cytokines that mediate the allergic response, we propose to use legendplex analysis. We estimate that all these antibody reagents may cost up to $1000.

D. OTHER EXPENSES

To evaluate the efficacy of our engineered therapeutics, we will test their ability to ameliorate hallmarks of allergic airway inflammation in mouse models of airway disease. Because of this, we have allocated $5,000 for animal purchase, and an additional $5,000 for per diem animal maintenance and care costs.

Program Director/Principal Investigator (Last, First, Middle): Eisenbarth, Stephanie

DETAILED BUDGET FOR INITIAL BUDGET PERIOD
DIRECT COSTS ONLY

FROM 6/1/23 THROUGH 5/31/24

List PERSONNEL *(Applicant organization only)*
Use Cal, Acad, or Summer to Enter Months Devoted to Project
Enter Dollar Amounts Requested *(omit cents)* for Salary Requested and Fringe Benefits

NAME	ROLE ON PROJECT	Cal. Mnths	Acad. Mnths	Summer Mnths	INST.BASE SALARY	SALARY REQUESTED	FRINGE BENEFITS	TOTAL
Stephanie Eisenbarth	PD/PI	.24			454,195	9,084	2,534	11,618
Adam Williams	Co-I	.12			180,778	1,808	504	2,312
Assel B.	Research As	2.4			74,990	14,998	4,184	19,182
SUBTOTALS →						25,890	7,222	33,112

CONSULTANT COSTS	
EQUIPMENT *(Itemize)*	
SUPPLIES *(Itemize by category)*	
Reagents - $8058	8,058
TRAVEL	
INPATIENT CARE COSTS	
OUTPATIENT CARE COSTS	
ALTERATIONS AND RENOVATIONS *(Itemize by category)*	
OTHER EXPENSES *(Itemize by category)*	

CONSORTIUM/CONTRACTUAL COSTS	DIRECT COSTS	
SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD *(Item 7a, Face Page)*		$ 41,170
CONSORTIUM/CONTRACTUAL COSTS	FACILITIES AND ADMINISTRATIVE COSTS	
TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD		$ 41,170

Program Director/Principal Investigator (Last, First, Middle): Eisenbarth, Stephanie

BUDGET FOR ENTIRE PROPOSED PROJECT PERIOD
DIRECT COSTS ONLY

BUDGET CATEGORY TOTALS	INITIAL BUDGET PERIOD *(from Form Page 4)*	2nd ADDITIONAL YEAR OF SUPPORT REQUESTED	3rd ADDITIONAL YEAR OF SUPPORT REQUESTED	4th ADDITIONAL YEAR OF SUPPORT REQUESTED	5th ADDITIONAL YEAR OF SUPPORT REQUESTED
PERSONNEL: *Salary and fringe benefits. Applicant organization only.*	33,112	34,105			
CONSULTANT COSTS					
EQUIPMENT					
SUPPLIES	8,058	8,058			
TRAVEL					
INPATIENT CARE COSTS					
OUTPATIENT CARE COSTS					
ALTERATIONS AND RENOVATIONS					
OTHER EXPENSES					
DIRECT CONSORTIUM/ CONTRACTUAL COSTS					
SUBTOTAL DIRECT COSTS *(Sum = Item 8a, Face Page)*	41,170	42,163			
F&A CONSORTIUM/ CONTRACTUAL COSTS					
TOTAL DIRECT COSTS	41,170	42,163			

TOTAL DIRECT COSTS FOR ENTIRE PROPOSED PROJECT PERIOD	**$** 83,333

JUSTIFICATION. Follow the budget justification instructions exactly. Use continuation pages as needed.

Dr. Stephanie Eisenbarth, MD, PhD; Principal Investigator (0.60 calendar months [5%])
Dr. Eisenbarth is a Professor and Chief of the Division of Allergy and Immunology at Northwestern University. She will be responsible for the conception, planning and design of the experiments. Dr. Eisenbarth will also coordinate the collaboration with The University of Chicago.

Adam Williams, Ph.D., Co-Investigator, (0.12 calendar months [1%])
Dr. Williams in an Associate Professor in the Division of Allergy and Immunology at Northwestern University and expertise in the fields of gene regulation, epigenetics, and type 2 immunity. Dr. Williams' lab space is contiguous with the lab of Dr. Eisenbarth. He will advise on molecular characterization of the allergic airway disease phenotypes in the proposed project.

Assel Biyasheva, Research Associate, (2.4 calendar months [20%]): Salary support is requested for a Research Associate. They will help with mouse experimental models.

Employee benefits have been calculated based on the following rates:
09/01/22 – 08/31/23…. 27.9%
09/01/23 – 08/31/24 and thereafter…. 27.9% (provisional)

SUPPLIES - $16,116 over 2 years
Money is requested to evaluate airway eosinophilia by bronchoalveolar lavage (BAL) using flow cytometry and cytospin, to quantify levels of serum IgG1 and IgE levels by ELISA, and the histological evaluation of mucus hyper secretion in the lungs by Periodic acid Schiff staining.

No F&A has been calculated for this project

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Jeffrey A Hubbell

eRA COMMONS USER NAME (credential, e.g., agency login): hubbell

POSITION TITLE: Eugene Bell Professor of Tissue Engineering, Professor of Molecular Engineering

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Kansas State University, Manhattan, KS	BS	05/1982	Chemical Engineering
Rice University, Houston, TX	PhD	05/1986	Chemical Engineering

A. Personal Statement

Jeffrey A. Hubbell is a highly experienced researcher in the field of molecular bioengineering, with research foci in regenerative medicine [1] and engineering immunity [2, 3]. Trained as a chemical engineer, he utilizes biomaterials engineering and protein engineering methodology to engineer growth factor/ extracellular matrix therapeutics in regenerative medicine as well as to engineer nanoparticle vaccines, tolerogenic (inverse) vaccines and targeted drug delivery vehicles. In the context of immunoengineering, he has developed novel technology by which to target antigens that are resident in the lymph nodes that drain the injection site. In work funded by the Gates Foundation, applications have focused on influenza and tuberculosis. In other work, application has focused on cancer vaccination. In the field of diabetes, Hubbell works in cell encapsulation for immunoisolation, in materials to induce peritransplant angiogenesis, and in antigen delivery approaches for antigen-specific tolerization.

Hubbell has been active in technology transfer, having had one technology to enter a variety of clinical products (through a start-up Focal Inc., now a part of Sanofi-Aventis, Focal having been acquired by Genzyme), another that has been in >500 patients in clinical trials and is still in clinical development (through a start-up Kuros Biosurgery, located in Zurich), another that is just entering clinical testing in the field of immunological tolerance (thorough Anokion, located in Lausanne, Switzerland and Cambridge, Mass), one that is in pre-clinical development in the field of food allergy (though ClostraBio, located in Chicago), and one that is in pre-clinical development in the field of immuno-oncology (through Arrow Immune, located in Chicago).

After 7 years at the University of Texas and 3 years at Caltech, Hubbell has spent the years of 1997 - 2014/16 in Switzerland, with a position from 2003 as Professor of Bioengineering and Chemical Engineering at the Ecole Polytechnique Fédérale de Lausanne (EPFL). While in Lausanne, he was founding Director of the Institute for Bioengineering and later Dean a.i. of the School of Life Sciences. In Summer 2014, he transitioned part-time from the EPFL to the University of Chicago, fully transitioning to Chicago in July 2016 as the Eugene Bell Professor of Tissue Engineering in the Pritzker School of Molecular Engineering. He is also a member of the University's Committee on Immunology, which functions as an interdepartmental immunology center, and the University's Committee on Cancer Biology, as well as the University of Chicago Comprehensive Cancer Center.

Hubbell recently completed NIH DP3 DK108215, Engineering Growth Factor-ECM Interactions to Heal Chronic Wounds in Type 1 Diabetes. This project developed variants of VEGF, PDGF-BB, and HB-EGF that were engineered to bind to extracellular matrix and fibrin as a provisional matrix in the wound microenvironment, an approach that led to enhanced wound healing in NOD Type 1 diabetic mice. This work is now being evaluated in large animals under sponsorship of MorphoGene SA, to move toward clinic. Hubbell is currently continuing

the project under sponsorship of MorphoGene.

Hubbell recently completed a project sponsored by AbbVie, Immunotherapy through Tumor Stroma-Targeting Nanoadjuvants. Here, his group developed polymers that bind in the tumor microenvironment and provide bio-molecular signals for both adjuvancy and tumor antigen uptake in the tumor microenvironment.

Hubbell is currently conducting a project with Anokion, Targetign LSECtin for Tolerogenic Delivery and for Induction of Humoral Tolerance to Foreign Proteins. Here, he is developing novel polymeric glycovectors for delivering autoimmune and protein drug antigens to liver sinusoidal endothelial cells for induction of immune tolerance. The approach, invented in the Hubbell laboratory, is now in Phase 1 clinical trials in celiac disease and multiple sclerosis, being developed by Anokion, Inc.

Ongoing and recently completed projects that I would like to highlight include:

R01 CA253248, “Probing cellular, molecular and biomechanical barriers to immunotherapy”, MA Swartz (PI), Role: coinvestigator, 08/01/21 – 07/31/26

R01 CA219304, “Paradoxical roles of tumor lymphangiogenesis on immunotherapy”, MA Swartz (PI), Role: coinvestigator, 04/01/18 – 03/30/23

Chicago Biomedical Consortium, “Vaccine efficacy against SARS-COV-2”, 08/01/20 – 07/31/22, JA Hubbell (co-PI) and MA Swartz (co-PI)

AbbVie-UChicago Program in Oncology, “Cancer Vaccines through Cell-surface Association of Vaccinal Antigens”, JA Hubbell (PI), 09/01/21 – 08/31/26

Chicago Immunoengineering Innovation Center, JA Hubbell (co-Director), providing funding for exploratory projects in immunoengineering with a view toward technology development and translation.

Citations:

1. Lutolf, M. & **Hubbell, J.** Synthetic biomaterials as instructive extracellular microenvironments for morphogenesis in tissue engineering. *Nature Biotechnology* **23**, 47-55 (2005).
2. Kontos, S., Grimm, A.J. & **Hubbell, J.A.** Engineering antigen-specific immunological tolerance. *Curr Opin Immunol* **35**, 80-88 (2015).
3. Hubbell, J.A., Thomas, S.N. & Swartz, M.A. Materials engineering for immunomodulation. *Nature* **462**, 449-460 (2009).

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2018 – Present	Co-Director, Chicago Immunoengineering Innovation Center, University of Chicago
2018 – Present	Committee on Cancer Biology, University of Chicago
2014/16 – Present	Committee on Immunology, University of Chicago
2014/16 – Present	Eugene Bell Professor of Tissue Engineering, Pritzker School of Molecular Engineering, University of Chicago
2014/16 – Present	Deputy Dean for Development, Pritzker School of Molecular Engineering, University of Chicago
2003 – Present	Professor of Bioengineering in the Institute of Bioengineering (Faculty of Life Sciences and Faculty of Engineering Sciences) and the Institute of Chemical Sciences and Technology (Faculty of Basic Sciences), Ecole Polytechnique Fédérale de Lausanne

2011 – 2013	Dean a.i., School of Life Sciences (http://sv.epfl.ch), Ecole Polytechnique Fédérale de Lausanne
2003 – 2011	Director, Institute of Bioengineering (http://ibi.epfl.ch), Ecole Polytechnique Fédérale de Lausanne
1997 – 2003	Professor of Biomedical Engineering in the Department of Materials (ETH) and the Fac. Medicine (University of Zurich); Director, Inst. Biomedical Engineering (ETH and Univ. Zurich)
1995 – 1997	Professor of Chemical Engineering, California Institute of Technology
1991 – 1994	Associate Professor of Chemical Engineering, University of Texas
1986 – 1991	Assistant Professor of Chemical Engineering, University of Texas

Honors

2021	Elected to the American Academy of Arts and Sciences, USA
2019	Elected to the National Academy of Medicine, USA
2014	Elected to the National Academy of Inventors, USA
2010	Elected to National Academy of Engineering, USA
2008	Alpha Chi Sigma Award, American Institute of Chemical Engineers
2008	Food, Pharmaceutical and Bioengineering Award, American Institute of Chemical Engineers
2008	Merck-Serono Chair in Drug Delivery
2006	George Winter Award, European Society for Biomaterials
2004	Gaden Award, John Wiley and Sons, and the American Chemical Society
2002	Körber Foundation Award for European Science
2000	Elected Fellow of Biomaterials Science and Engineering
2000	Elected Fellow of the American Association for the Advancement of Science
1996	Clemson Award for Applied Research, Society for Biomaterials
1995	Elected Fellow, American Institute of Medical and Biological Engineering

C. Contributions to Science

Hubbell's scientific contributions have been in the fields of regenerative medicine and immunoengineering. These contributions are summarized below:

I. Regenerative Medicine – bioactive biomaterials: In the biomaterials community, it is now very common to build in synthetic or biosynthetic groups such as peptides to provide adhesion sites or protease substrate sites to provide cell-driven proteolysis. Hubbell was the first to make these innovations, which have now been widely adopted. In the early 1990s, he first introduced the concept of incorporation of synthetic peptide adhesion ligands [4], which he use in studies of the cell biology of adhesion and in vascular biology applications. In the early 2000s, he first introduced the concept of biomaterials engineered to be remodeled by cell-derived proteases, which he used in studies of bone repair [5] and engineered angiogenesis [6]. Current implementations of these materials now recapitulate most of the characteristics of the natural extracellular matrix: receptor-mediated adhesion, protease-mediated remodeling, and bioaffinity-based growth factor incorporation [7].

4. Massia, S. & **Hubbell, J**. An RGD Spacing Of 440nm Is Sufficient For Integrin Alpha-V-Beta-3-Mediated Fibroblast Spreading And 140nm For Focal Contact And Stress Fiber Formation. The Journal of cell biology 114, 1089-1100 (1991).
5. Lutolf, M., Weber, F., Schmoekel, H., Schense, J., Kohler, T., Muller, R. & **Hubbell, J.** Repair of bone defects using synthetic mimetics of collagenous extracellular matrices. *Nature Biotechnology* **21**, 513-518 (2003).
6. Zisch, A.H., Lutolf, M.P., Ehrbar, M., Raeber, G.P., Rizzi, S.C., Davies, N., Schmokel, H., Bezuidenhout, D., Djonov, V., Zilla, P. & **Hubbell, J.A.** Cell-demanded release of VEGF from synthetic, biointeractive cell ingrowth matrices for vascularized tissue growth. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* **17**, 2260-2262 (2003).
7. Martino, M.M., Briquez, P.S., Ranga, A., Lutolf, M.P. & **Hubbell, J.A.** Heparin-binding domain of fibrin(ogen) binds growth factors and promotes tissue repair when incorporated within a synthetic

matrix. Proceedings of the National Academy of Sciences of the United States of America 110, 4563-4568 (2013).

II. Regenerative Medicine – engineered growth factors: Starting in the late 1990s, Hubbell began engineering adhesion peptides [8] and growth factors for binding to extracellular matrices such as fibrin. He invented an approach where he fused recombinant growth factors to a domain of the protein α2-plasmin inhibitor that binds covalently to fibrin during fibrinogen polymerization under the influence of factor XIIIa [8]. Over the years, a number of such growth factors were engineered in the context of bone repair [9] and induction of angiogenesis [10], showing qualitative and quantitative benefits over use of the wild-type parent growth factors. Hubbell started Kuros Biosurgery AG to develop this technology. The products of this research have now been in in clinical trials in enhancement of skin graft 'take' (Phase IIb successfully completed; Kuros), in enhancement of healing in soft tissue plastic surgery (Phase IIb successfully completed; Kuros), in promotion of bone repair in the tibial plateau (Phase IIb successfully completed in 180 patients; Kuros), and in repair of bone repair in open tibial fractures (in Phase IIb, successfully completed in approx. 200 patients; Kuros). Work in bone repair is moving to Phase III evaluation. In this work, an entirely new approach to morphogen delivery was evaluated, and two new biomolecules, designed by Hubbell and his group, were taken to randomized and controlled clinical evaluation).

8. Schense, J., Bloch, J., Aebischer, P. & **Hubbell, J.** Enzymatic incorporation of bioactive peptides into fibrin matrices enhances neurite extension. *Nature Biotechnology* **18**, 415-419 (2000).
9. Arrighi, I., Mark, S., Alvisi, M., von Rechenberg, B., **Hubbell, J.A**. & Schense, J.C. Bone healing induced by local delivery of an engineered parathyroid hormone prodrug. *Biomaterials* **30**, 1763-1771 (2009).
10. Ehrbar, M., Djonov, V., Schnell, C., Tschanz, S., Martiny-Baron, G., Schenk, U., Wood, J., Burri, P., **Hubbell, J**. & Zisch, A. Cell-demanded liberation of VEGF(121) from fibrin implants induces local and controlled blood vessel growth. *Circulation Research* **94**, 1124-1132 (2004).

III. Regenerative Medicine – extracellular matrix determination of growth factor function: In studies from around 2010 to present that are both fundamental and translational, Hubbell identified the broad capacity for ECM molecules to bind to and synergize with growth factors. For example, he demonstrated that fibronectin can bind a host of growth factors leading to synergistic signaling between integrins and the corresponding growth factor receptor [11]. His group also identified a promiscuous binding site in fibrinogen/fibrin [7] and tenascin C. These observations were used to engineer variant forms of growth factors that can bind with super-affinity to ECM proteins, which he demonstrated in models of bone and skin repair [12]. He has also gone on to investigate the axis between ECM signaling and innate and adaptive immunity [13].

7. Martino, M.M., Briquez, P.S., Ranga, A., Lutolf, M.P. & **Hubbell, J.A.** Heparin-binding domain of fibrin(ogen) binds growth factors and promotes tissue repair when incorporated within a synthetic matrix. Proceedings of the National Academy of Sciences of the United States of America 110, 4563-4568 (2013).
11. Martino, M.M., Tortelli, F., Mochizuki, M., Traub, S., Ben-David, D., Kuhn, G.A., Müller, R., Livne, E., Eming, S.A. & **Hubbell, J.A.** Engineering the growth factor microenvironment with fibronectin domains to promote wound and bone tissue healing. Science translational medicine 3, 100ra189 (2011).
12. Martino, M.M., Briquez, P.S., Guc, E., Tortelli, F., Kilarski, W.W., Metzger, S., Rice, J.J., Kuhn, G.A., Muller, R., Swartz, M.A. & **Hubbell, J.A.** Growth factors engineered for super-affinity to the extracellular matrix enhance tissue healing. Science 343, 885-888 (2014).
13. Julier, Z., Martino, M.M., de Titta, A., Jeanbart, L. & **Hubbell, J.A**. The TLR4 agonist fibronectin extra domain A is cryptic, exposed by elastase-2; use in a fibrin matrix cancer vaccine. Sci Rep 5, 8569 (2015).

IV. Immunoengineering – proteins and nanomaterials as lymph node-targeting vaccines and protein engineering in cancer immunotherapy: Biomaterials developments from the mid-2000s to present in Hubbell's laboratory include the design, synthesis and biological evaluation of novel block copolymers that degrade by either oxidative or reductive mechanisms. These polymers, formulated as ultra-small (ca. 20 nm) polymeric nanoparticles have been successfully applied as a promising vaccine platform

technology in collaboration with the laboratory of Melody Swartz at EPFL [14]. These materials attracted substantial attention in the academic vaccine community as well as in industry after successful application in targeting the lymphatic circulation, and they are currently being pursued with approx. $7 million in funding from the European Commission and the Bill and Melinda Gates Foundation in HIV (Gates), TB (Gates), chronic hepatitis B (EC), and influenza (EC). Although presently in studies in rodents, protection against challenge in influenza is very promising, and protection against challenge with TB also promising. Current work addresses antigen-nonspecific approaches in development of immunity to fight cancer. Our first efforts have been in tumor-retained checkpoint immunotherapy antibodies [15] and in tumor-retained immune agonist antibodies [16]. We have very recently extended this approach to target antibodies (demonstrated with αCTLA4 and αPD-L1) and cytokines to tumors (demonstrated with IL-2[17]), in addition to chemokines (in preparation). In these works, we have demonstrated that our protein engineering approach can both enhance efficacy and reduce immune-related adverse events.

14. Reddy, S.T., van der Vlies, A.J., Simeoni, E., Angeli, V., Randolph, G.J., O'Neil, C.P., Lee, L.K., Swartz, M.A. & **Hubbell, J.A.** Exploiting lymphatic transport and complement activation in nanoparticle vaccines. *Nature Biotechnology* **25**, 1159-1164 (2007).
15. Ishihara, J., Fukunaga, K., Ishihara, A., Larsson, H.M., Potin, L., Hosseinchi, P., Galliverti, G., Swartz, M.A. & **Hubbell, J.A.** Matrix-binding checkpoint immunotherapies enhance antitumor efficacy and reduce adverse events. *Science translational medicine* **9**, eaan0401 (2017).
16. Ishihara, J., Ishihara, A., Potin, L., Hosseinchi, P., Fukunaga, K., Damo, M., Gajewski, T.F., Swartz, M.A. & **Hubbell, J.A.** Improving Efficacy and Safety of Agonistic Anti-CD40 Antibody Through Extracellular Matrix Affinity. *Mol Cancer Ther*, molcancerther0091.2018 (2018).
17. Ishihara, J., Ishihara, A., Sasaki, K., Lee, S.S., Williford, J.M., Yasui, M., Abe, H., Potin, L., Hosseinchi, P., Fukunaga, K., Raczy, M.M., Gray, L.T., Mansurov, A., Katsumata, K., Fukayama, M., Kron, S.J., Swartz, M.A. & **Hubbell, J.A.** Targeted antibody and cytokine cancer immunotherapies through collagen affinity. *Science translational medicine* **11** (2019).

V. Immunoengineering – antigen delivery in immunological tolerance: The latest protein engineering development in Hubbell's laboratory are in the domain of inverse vaccination, i.e. antigen-specific therapies to establish immunological tolerance. He invented an approach to target antigens in a tolerogenic manner to antigen-presenting cells resident in the liver, resulting in antigen-specific tolerization of T cells that are reactive to that antigen [18, 19]. He has shown this in the context of tolerance to protein drugs in mouse models [20], as well as in tolerance induction in the NOD mouse model of autoimmune type 1 diabetes mellitus and in the EAE model of multiple sclerosis[21]. He founded a company, Anokion SA (Lausanne, Switzerland, and Cambridge, Mass.) that has raised approx. $100 million to develop the technology; this company is now partnered with Bristol Myers Squibb and has entered clinical trials in 2020 in a celiac disease trial.

18. Grimm, A.J., Kontos, S., Diaceri, G., Quaglia-Thermes, X. & **Hubbell, J.A.** Memory of tolerance and induction of regulatory T cells by erythrocyte-targeted antigens. *Sci Rep* **5**, 15907 (2015).
19. Kontos, S., Kourtis, I.C., Dane, K.Y. & **Hubbell, J.A.** Engineering antigens for in situ erythrocyte binding induces T-cell deletion. *Proceedings of the National Academy of Sciences of the United States of America* **110**, E60-68 (2013).
20. Lorentz, K.M., Kontos, S., Diaceri, G., Henry, H. & **Hubbell, J.A.** Engineered binding to erythrocytes induces immunological tolerance to E. coli asparaginase. *Science Advances* **1** (2015).
21. Wilson, D.S., Damo, M., Hirosue, S., Raczy, M.M., Brunggel, K., Diaceri, G., Quaglia-Thermes, X. & **Hubbell, J.A.** Synthetically glycosylated antigens induce antigen-specific tolerance and prevent the onset of diabetes. *Nat Biomed Eng* 3, 817-829 (2019).

Link to My Bibliography on Pubmed:
http://www.ncbi.nlm.nih.gov/sites/myncbi/1r7pyUO_0umQJ/bibliography/40417024/public/?sort=date&direction=ascending

ISI Web of Science (as of November 2021): ISI H-index = 114, Total citations > 45,500, Citations per year approx. 2500

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Eisenbarth, Stephanie Caroline

eRA COMMONS USER NAME (credential, e.g., agency login): seisenbarth

POSITION TITLE: Professor and Chief, Allergy & Immunology

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Bryn Mawr College	BA	06/1996	Chemistry
Yale University, New Haven, CT	PhD	05/2003	Immunobiology
Yale University, New Haven, CT	MD	05/2005	Medicine
Yale University, New Haven, CT	Resident	05/2008	Laboratory Medicine
Yale University, New Haven, CT	Fellow	05/2010	Immunobiology

A. Personal Statement

My research group is focused on understanding the mechanisms driving allergy, a field I have worked in for two decades. Utilizing clinical data and human samples to guide our studies and mouse models to test new mechanistic paradigms, we have identified novel and unexpected immune cell subsets and functions. Our recent work has focused on the functions of T follicular helper (Tfh) cells. The cytokine profile of Tfh cells contributes to their ability to promote particular antibody isotype production by B cells. Recently, we identified a new Tfh subset with an atypical cytokine profile that is necessary for inducing high affinity IgE responses to allergens, challenging prior assumptions about how IgE is regulated and functional possibilities of Tfh cell subsets. For the purpose of this proposal, we will help we will test the therapeutic efficacy of the Gas6-allergen fusion proteins and the pMan-conjugated allergens at ameliorating disease in mouse models of allergic airway inflammation. We have significant experience with the relevant models required for this project and this work will be a natural extension of the existing work in my lab.

1. Soldatenko A, Hoyt LR, Xu L, Calabro S, Lewis SM, Gallman AE, Hudson KE, Stowell SR, Luckey CJ, Zimring JC, Liu D, Santhanakrishnan M, Hendrickson JE, **Eisenbarth SC**. Innate and Adaptive Immunity to Transfused Allogeneic RBCs in Mice Requires MyD88. *J Immunol.* Jan 2022
2. Yin, X., Chen, S., **Eisenbarth SC**. Dendritic Cell Regulation of T Helper Cells. *Annu Rev Immunology* 2021 April; 39(1):759-790. doi.org/10.1146/annurev-immunol-101819-025146
3. Calabro S, Liu D, Gallman A, Nascimento MS, Yu Z, Zhang TT, Chen P, Zhang B, Xu L, Gowthaman U, Krishnaswamy JK, Haberman AM, Williams A, **Eisenbarth SC**. Differential intrasplenic migration of dendritic cell subsets tailors adaptive immunity. *Cell Rep.* 2016 Aug 30;16(9):2472-85. PMID: 27545885; PubMed Central PMCID: PMC6323650
4. Calabro S, Gallman A, Gowthaman U, Liu D, Chen P, Liu J, Krishnaswamy JK, Nascimento MS, Xu L, Patel SR, Williams A, Tormey CA, Hod EA, Spitalnik SL, Zimring JC, Hendrickson JE, Stowell SR, **Eisenbarth SC**. Bridging channel dendritic cells induce immunity to transfused red blood cells. *J Exp Med.* 2016 May 30;213(6):887-96. PubMed PMID: 27185856; PubMed Central PMCID: PMC4886363

Current Projects

R01 AI136942
NIAID/NIH **Eisenbarth (PI)** 09/2018 – 08/2023
"Immune mechanisms regulating allergy"
The goal of these studies is to define the nature of the T cell that induces allergen-specific IgE using aeroallergen sensitization models

FARE Mid Stage Investigator in Food Allergy Award **Eisenbarth (PI)** 07/2017-03/2023 (NCE)
"Role of Dock8 in Maintaining Tolerance to Food Antigens"

The goal of this grant is to identify novel immune pathways regulated by DOCK8 that predispose individuals to food allergy.

R56AI155497

NIH/NIAID **Eisenbarth (PI)** 08/2021-07/2023 (NCE)

“The adaptive immune response to food antigens in the gut”

The goal of this study is to define nechanisms of allergen-specific IgE versus IgA induction in the gut.

Food Allergy Fund **Eisenbarth/Williams (PI)** 07/2022-06/2023

“Novel mechanisms of blocking anaphylaxis to food allergens”

The studies outlined in this proposal will enable the development of new diagnostic and therapeutic approaches to improve management of those with food allergy.

2023-FASI-9394

Food Allergy Science Initiative, Inc **Eisenbarth (PI)** 07/2022-06/2023

“Preventing Anaphylaxis After Sensitization to Food”

In this proposal we will evaluate two pathways that might prevent the interaction of a food allergen with a sensitized mast cell.

R01AI162645

NIH/NIAID (via subcontract from Yale University) **Wang (PI), Eisenbarth (Sub PI)** 05/2022-04/2026

“Dissecting How Xenobiotics Act as Adjuvants for Oral Allergic Sensitization”

The proposed research will mechanistically dissect how NSAIDs act as adjuvants to cause allergic sensitization.

R01AI153344

NIH/NIAID **Williams (PI), Eisenbarth (Co-I)** 09/2021 - 08/2026

“Long Non-Coding RNAs in Allergy”

The overall goal of this proposal is to identify fundamental mechanisms controlling allergic responses by the long non-coding RNA (lncRNA) Morrbid

Pending Projects

R01AI177532

NIH/NIAID **Eisenbarth/Williams (PI)** 07/2023-06/2028

“Determinants of oral anaphylaxis to food”

The goal of this proposal is to identify new ways of preventing anaphylaxis in those with food allergy by defining cellular and molecular mechanisms that transport intact food allergens across the gut epithelium.

R01HL164723

NIH **Eisenbarth (PI)** 07/2023-06/2028

“Innate immune triggers of alloantibody induction”

We will determine TLR-dependent activation pathways in DCs and B cells necessary for alloimmunization and will trial validated TLR inhibitors to see if we can avert alloimmunization in our mouse model.

G. Harold & Leila Y. Mathers Foundation **Eisenbarth (PI)** 01/2023-12/2025

“Establishing the immune structure of the human spleen with spatial profiling”

The ultimate goal is to understand how the human immune system in the spleen is responding to transfused red blood cells in patients who are transfusion dependent, including those with sickle cell disease (SCD).

R01AI177313

NIH **Eisenbarth/Williams (PI)** 07/2023-06/2028

“Regulation of T Cell Metabolism”

The proposed studies will define how DOCK8 regulates metabolic reprogramming and Th2 polarization and will test whether these programs are integrated by DOCK8

NIH/NIAID **Dulai (PI), Eisenbarth (Co-I)** 03/2023-02/2028

“Defining Ulcerative Colitis Endotypes and Immune Cell Drivers of Disease Development”

We aim to fully define the spectrum of immune and epithelial cell heterogeneity and endotypes in UC and determine whether this endotype based classification successfully predicts response to pathway specific biologics or small molecule therapies

NIH **Abdala-Valencia (PI), Eisenbarth (minor user)**

“CosMx Spatial Molecular Imager” 02/2023-01/2024

The CosMx Spatial Molecular Imaging Platform (CosMxTM SMI, NanoString, Seattle, WA) will allow a well-funded group of biomedical scientists experienced in the application of single cell transcriptomic approaches to human disease to perform combined spatial transcriptomics and proteomics at a cellular and subcellular resolution using tissue samples collected at Northwestern University (NU) and within the greater Chicago area.

B. Positions, Scientific Appointments and Honors

Positions and Appointments

2022-Pres.	Professor of Medicine, Chief of Allergy and Immunology & Director of the Center for Human Immunobiology, Northwestern University Feinberg School of Medicine
2020-2021	Associate Professor with Tenure, Department of Laboratory Medicine, Department of Immunobiology, Section of Rheumatology, Allergy & Immunology, Yale School of Medicine
2018-2021	Associate Chair of Research, Department of Laboratory Medicine, Yale School of Medicine
2017-2021	Assistant Director Clinical Pathology Residency Training Program, Yale-New Haven Hospital
2009-2021	Assistant Director Clinical Immunology Laboratory, Yale-New Haven Hospital
2017-2020	Associate Professor, Yale School of Medicine
2011-2017	Assistant Professor, Yale School of Medicine
2010-2011	Instructor, Yale School of Medicine
2007-2010	Fellow, Department of Immunobiology, Yale School of Medicine, Laboratory of Richard Flavell
2005-2008	Resident, Clinical Pathology, Department of Laboratory Medicine, Yale-New Haven Hospital
1996-2005	Medical Scientist Training Program, Yale School of Medicine, Laboratory of Kim Bottomly

Other Experience and Professional Membership

2022 - Pres.	Editorial Board, *Journal of Clinical Investigation*
2022 - Pres.	Editorial Advisor, *Science Immunology*
2020 - Pres.	Editorial Committee, *Annual Review of Pathology: Mechanisms of Disease*
2020 - Pres	Chair education committee, Federation of Clinical Immunology Societies (FOCIS)
2008 - Pres.	Board Certified in Clinical Pathology, American Board of Pathology

Honors

2021	Top 100 Women in Food Allergies by Spokin
2020	Election to the American Society of Clinical Investigation (ASCI)
2017	National Blood Foundation Annual Award for Innovative Research
2016	Ellis Benson Award for a young faculty member in recognition of meritorious accomplishments, Academy of Clinical Laboratory Physicians & Scientists (ACLPS)
2014	Young Physician-Scientist Award, The American Society for Clinical Investigation (ASCI)
2014	G. Jeanette Thorbecke Award for an outstanding early career female scientist, Society for Leukocyte Biology (SLB)
2013	Clinical Scientist Development Award, Doris Duke Charitable Foundation
2012	Women & Diversity paper of the year, Society for Leukocyte Biology (SLB)

C. Contributions to Science

1. Uncovering immunologic mechanisms of alloimmunity to transfused red blood cells

Red blood cell (RBC) transfusion therapy is essential for the survival of patients with hematological disorders and bone marrow failure syndromes such as sickle cell anemia and myelodysplastic syndrome. As a clinical pathologist, I see the potentially life-threatening complication of RBC transfusion – the development of non-ABO alloantibodies. My lab was the first to demonstrate that a subset of conventional DCs in the spleen is necessary for CD4+ T cell priming to RBC-derived alloantigens and to test for the innate immune receptors that detect transfused RBCs and activate DCs. This has important implications for preventing a potentially harmful consequence of transfusion, in particular in patients requiring lifelong RBC transfusion support. These fundamental studies also elucidated the cellular interactions and organization in the spleen that enable adaptive immunity to systemic antigens.

1. Soldatenko A, Hoyt LR, Xu L, Calabro S, Lewis SM, Gallman AE, Hudson KE, Stowell SR, Luckey CJ, Zimring JC, Liu D, Santhanakrishnan M, Hendrickson JE, **Eisenbarth SC**. Innate and Adaptive Immunity to Transfused Allogeneic RBCs in Mice Requires MyD88. *J Immunol.* Jan 2022
2. Lewis, SM, Williams, A., **Eisenbarth, SC**. Structure and function of the immune system in the spleen. *Science Immunology* 2019. PubMed PMID:30824527 PubMed Central PMCID:PMC6495537

3. Calabro S, Gallman A, Gowthaman U, Liu D, Chen P, Liu J, Krishnaswamy JK, Nascimento MS, Xu L, Patel SR, Williams A, Tormey CA, Hod EA, Spitalnik SL, Zimring JC, Hendrickson JE, Stowell SR, **Eisenbarth SC**. Bridging channel dendritic cells induce immunity to transfused red blood cells. *J Exp Med.* 2016 May 30;213(6):887-96. PubMed PMID:27185856; PubMed Central PMCID:PMC4886363.
4. Gibb DR, Calabro S, Liu D, Tormey CA, Spitalnik SL, Zimring JC, Hendrickson JE, Hod EA, **Eisenbarth SC**. The Nlrp3 Inflammasome Does Not Regulate Alloimmunization to Transfused Red Blood Cells in Mice. *EBioMedicine.* 2016 Jul;9:77-86. PubMed Central PMCID: PMC4972549.

2. Cellular mechanisms of allergic inflammation

My early work identified an essential role for innate immune instruction for aeroallergen sensitization. This represented a paradigm shift in the asthma field, and our *J. Exp. Med.* paper has been cited over 1000 times. More recent work from my lab has advanced our understanding of how antibody responses to allergens are regulated. We identified a new type of T follicular helper (Tfh) cell—Tfh13—that drives IgE responses to allergens, expanding the possible functional roles of Tfh cell subsets. In contrast, we found that Tfh cells are not required for production of IgA antibodies in the gut. This regulatory distinction has important implications for redirecting the antibody response to food allergens. Our most recent work has uncovered an unexpected link between antigen uptake from the GI track and susceptibility to anaphylaxis in one of the few mouse models that demonstrate oral anaphylaxis, C3H/HeJ. We are actively pursuing the cellular mechanism and genetic underpinnings of this phenotype.

1. Gertie JA, Zhang G, Liu EG, Hoyt LR, Yin X, Xu L, Long LL, Soldatenko A, Gowthaman U, Williams A*, **Eisenbarth SC***. Oral anaphylaxis to peanut in a mouse model is associated with gut permeability but not with *Tlr4* or *Dock8* mutations. *J Allergy Clin Immunol*, Jan 2022 (*Co-corresponding); PMCID: PMC8626534

   **Editorial in JACI by T. Platts-Mills*
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3. Redefining how dendritic cell subsets direct distinct T cell responses

Dendritic cells (DCs) are the primary antigen-presenting cell for naïve T cells. It was known that subsets of DCs exist and that each subset has functional specialization. How this specialization was achieved remained unclear. We demonstrated that type 2 conventional DC (cDC2)–CD4+ T cell interactions happen in unique niches in both the splenic white pulp and lymph nodes. Our findings contribute to a new paradigm in which specialized DC subsets preferentially interact in domains enriched for CD4+ or CD8+ T cells to selectively promote particular adaptive immune responses.

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4. Defining the role of DOCK8 in cells of the immune system

My lab showed that the guanine nucleotide exchange factor, DOCK8 (dedicator of cytokinesis 8), was essential for the coordinated DC migration to draining lymph nodes, particularly for type 2 DCs (cDC2s). Using conditional deletion of DOCK8, we have furthered the understanding of how this guanine nucleotide exchange factor regulates the immune function of B cells and T cells.

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5. Immune mechanisms regulating antibody production to vaccination and infection

Vaccines are one of the most important medical interventions in global health. As most vaccines protect the host via induction of antibody production, we have worked to define the cellular pathways that result in efficient antibody induction following immunization and also infection. Work over the past decade has identified that a subset of T cells, known as T follicular helper (Tfh) cells, are required to promote long-lived, high-affinity antibody production by B cells. We have identified how different adjuvants stimulate DCs to prime Tfh cells. This includes our discovery that the NLRP3 inflammasome is part of a molecular pathway determining the immunogenicity of the most commonly used adjuvant in vaccines, aluminum hydroxide. More recently, we determined that $CD11b^{+}$ migratory type 2 conventional DCs (cDC2s) are necessary and sufficient for Tfh cell induction to immunization. These findings could help guide strategies to effectively deliver vaccines at lower doses during pandemics, when vaccine supplies are stretched. We have most recently studied whether the same cellular interactions are required for the antibody response to SARS-CoV-2. Surprisingly, our work suggests that multiple T cell populations support antibody responses to respiratory viruses.

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