BIOGRAPHICAL SKETCH

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NAME: Stehlik, Christian

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

POSITION TITLE: Professor

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
University of Vienna, Austria	MS	05/1994	Genetics & Immunology
University of Vienna, Austria	PhD	02/1999	Molecular Biology
University of Vienna, Austria	Post-Doc	07/1999	Inflammation
The Burnham Institute, La Jolla, CA	Post-Doc	082003	Inflammation

A. Personal Statement

I have a broad background in immunology, cell biology, molecular biology and biochemistry and my area of expertise is inflammatory signaling mechanisms in macrophages during inflammatory and infectious disease. My laboratory focuses on delineating the molecular mechanisms of the inflammatory host response following tissue damage and infection. A particular focus area is activation and signaling of cytosolic pattern recognition receptors of the Nod-like receptor (NLR) and AIM-2-like receptor (ALR) families and their contribution to homeostasis, host defense, inflammatory disease and autoimmunity through activation of inflammatory caspases. A main emphasis is on the molecular mechanisms that regulate activation and assembly of inflammasomes in macrophages and we are particularly interested in negative regulatory mechanisms that contribute to the resolution phase of inflammation. Our work now also focuses on inflammasomes and inflammasome responses in epithelial cells in the intestine and the lung. Work from my laboratory resulted in the discovery of several inflammasome-regulatory mechanisms: We identified the inflammasome adaptor ASC as the essential adaptor for caspase-1 activation **[2b]**, described the mechanism by which inflammasome formation is regulated by ASC adaptor protein availability **[4a]** and splicing **[4b]** and identified regulation of the non-canonical inflammasome **[4d]**. We discovered NLRP7 as a novel inflammasome-activating pattern recognition receptor in human macrophages and demonstrated its cooperation with TLR2 in the response to bacterial acylated lipopeptides and provided evidence of differences in inflammasome responses in human and mice **[3b,c]**. We discovered one of the Caspase recruitment domain (CARD)-only proteins, which inhibits caspase-1 and discovered all currently known full members of - and subsequently established the family of PYRIN domain-only proteins (POPs), as key regulators of inflammasomes and NF-κB **[5a-d]** and developed an NLRP3 inflammasome-targeting drug for inflammatory disease **[5b]**. My laboratory has a large collection of molecular tools, including expression constructs, recombinant adenovirus and lentiviruses, siRNAs and shRNAs and unique antibodies and established protocols to study innate immune signaling responses. We have knock-out mice for core inflammasome proteins and several unique conditional transgenic and knock-out mice for novel inflammasome and key innate immune adaptors. We established shRNA and CRISPR mediated KD/KO THP-1 cells and J2 virus immortalized KO iBMDM for most inflammasome components and key innate immune adaptors and have restored several as GFP-tagged proteins. We also developed an innovative caspase-1 reporter mouse to contribute to the successful completion of this study.

Most relevant publications to the current application

- de Almeida, L, Khare, S, Misharin, AV, Patel, R, Wallin, M, Perlman, HR, Greaves, DR, Hoffman, HM, Dorfleutner, A*, **Stehlik, C*** (2015) The PYRIN domain-only protein POP1 inhibits inflammasome assembly

and ameliorates inflammatory disease. *Immunity* 43, 264-276. PMC4666005. Highlighted with a Commentary by Shimada K Timothy R Crother TR, Arditi, M (2015) POPsicle for Fever! Cooling Down the Inflammasome. *Immunity*, 43, 213-215. *co-corresponding author

- Khare, S, Ratsimandresey, RA, de Almeida, L, Cuda, CM, Rellick, SL, Misharin, AV, Wallin, MC, Gangopadhyay, A, Forte, E, Gottwein, E, Perlman, H, Reed, JC, Greaves, DR, Dorfleutner, A*, **Stehlik, C*** (2014) The PYRIN domain-only protein POP3 inhibits AIM2-like receptor inflammasomes and regulates responses to DNA virus infections. *Nature Immunology,* 15, 343-345**,** PMC4123781. April Cover article, highlighted with a News and Views Commentary by Krishnaswamy JK, Liu D, Eisenbarth SC (2014) POP goes the inflammasome. *Nature Immunology*, 15, 311-313. *co-corresponding author

- Khare, S, Dorfleutner, A, Bryan, NB, L., Yun, C, Radian, AD, de Almeida, L, Rojanasakul, Y, **Stehlik, C** (2012) An NLRP7-containing inflammasome mediates recognition of microbial lipopeptides in macrophages. *Immunity*, 36, 464-476*.* PMC3315380. Highlighted in Nature Reviews Immunology 12, 3, Nature Immunology 13, p358 and by The Faculty of 1000.

- Chu, HL, Indramohan, M, Ratsaimandresey, RA, Gangopadhyay, A, Morris, EP, Monack, DM, Dorfleutner, A*, and **Stehlik, C*** (2018) The oxidized phospholipid oxPAPC protects from septic shock by targeting non-canonical inflammasome in macrophages, *Nature Communications,* 9, 996, PMC5843631. *co-corresponding author

B. Positions and Honors

Positions and Employment

1999	Postdoctoral Associate: Laboratory of J. Lipp, Department of Vascular Biology and Thrombosis Research, University of Vienna, Austria. (Inflammation and NF-κB)
1999-2003	Postdoctoral-Fellow: Laboratory of C. John Reed, Program on Apoptosis and Cell Death Research, The Burnham Institute, La Jolla, CA. (the Death Domain Fold in Innate Immunity)
2003-2006	Research Assistant Professor, Mary Babb Randolph Cancer Center and the Department of Microbiology, Immunology & Cell Biology, West Virginia University, Morgantown, WV.
2003-2007	Guest Researcher, Center of Disease Control/NIOSH/PPRB, Morgantown, WV
2006-2007	Assistant Professor, Mary Babb Randolph Cancer Center and the Department of Microbiology, Immunology & Cell Biology, West Virginia University, Morgantown, WV.
2007-2014	Assistant Professor (tenure track), Department of Medicine/Rheumatology, member of the Robert H. Lurie Comprehensive Cancer Center, Feinberg School of Medicine, Northwestern University, Chicago, IL. and John P. Gallagher Research Professor of Rheumatology
2008-2018	Member of the Interdepartmental Immunobiology Center, Northwestern University
2011-2018	Member of the Skin Disease Research Center, Northwestern University
2014-2017	Associate Professor (with tenure), Department of Medicine/Rheumatology, Northwestern U.
2015-2018	Director, Immunology and Microbial Sciences Graduate Training Program, Northwestern U.
2017-2018	Professor (with tenure), Department of Medicine/Rheumatology, Northwestern University
2018-	Professor, Department of Pathology and Laboratory Medicine, and Department of Biomedical Sciences, Cedars-Sinai Medical Center, Los Angeles, CA
2018-	Director for Pathology Research, Cedars-Sinai Medical Center, Los Angeles, CA

Other Experience and Professional Membership:

2005	NIH IDM-G90 (S) study section (ad hoc), NIH IDM-G 02 study section (ad hoc)
2006	NIH Innate Immunity and Inflammation (III) study section (ad hoc)
2007	NIH Innate Immunity and Inflammation (III) study section (ad hoc)
2008	Grant review for The Wellcome Trust and the The Broad Foundation
2009	NIH Innate Immunity and Inflammation (III) study section (ad hoc)
2010-	American Heart Association Immunology Bsc2 study section (member),
2010	Arthritis Foundation OA IRG Review Panel B (ad hoc), NIH Innate Immunity and Inflammation (III) study section (ad hoc), ATIP-Avenir Research program (ad hoc)
2011	NIH ZRG1 IMM-N (03) M study section (ad hoc), ATIP-Avenir Research program (ad hoc)
2012	Hungarian Scientific Research Fund (OTKA) (ad hoc)
2014-	NIH Innate Immunity and Inflammation (III) study section (member)
2017	NIH ZRG1 IMM-F (03) M study section (ad hoc), The Gottfried Wilhelm Leibniz Prize, German Research Foundation (external advisor)

Honors:

1994, 1999	Graduation with honors (M.S., PhD, respectively)
1999-2001	Austrian Science Foundation Postdoctoral Fellowship Awards (J1809-Gen and J1990-Gen)
2001	Department of Defense Breast Cancer Postdoctoral Fellowship Award (DAMD170110171)
2004	Faculty Development Award, West Virginia University
2007	Appointment to the John P. Gallagher Research Professor of Rheumatology
2012, 2014	AAI Early Career Faculty Travel Grants
2017	Driskill Graduate Program in Life Science (DGP) Faculty Service Award, Northwestern U.

C. Contributions to Science

1) My doctoral training focused on identifying genes that are differentially expressed during inflammation in endothelial cells. I discovered the 3 human BIR domain-containing inhibitor of apoptosis (IAP) genes (cIAP1, cIAP2 and XIAP) as TNFα-inducible genes, and demonstrated that they protect endothelial cells from TNFα-induced apoptosis during inflammation **[a, b, c]**. I further discovered a novel endothelial cell specific adhesion receptor, which is inducible expressed during inflammation **[d]**.

[a] **Stehlik, C**, de Martin, R, Binder, BR, Lipp, J. (1998). Cytokine induced expression of porcine Inhibitor of Apoptosis Protein (iap) family member is regulated by NF-κB. *Biochem. Biophys. Res. Com.* 234, 827-832.

[b] **Stehlik, C**, de Martin, R, Kumabashiri, I, Schmid, JA, Binder, BR, Lipp, J (1998). Nuclear Factor (NF)-kB-regulated X-chromosome-linked *iap* Gene Expression Protects Endothelial Cells from Tumor Necrosis Factor α-induced Apoptosis. *J. Exp. Med.* 188, 211-216, PMC2525542.

[c] Hofer-Warbinek, R, Schmid, JA, **Stehlik, C**, Binder, BR, Lipp, J, de Martin, R. (2000). Activation of NF-κB by XIAP, the X chromosome-linked inhibitor of apoptosis. *J. Biol. Chem*. 275, 22064-22068.

[d] **Stehlik C**, Kroismayr, R, Dorfleutner A, Binder BR, Lipp J (2004) VIGR – a novel inducible adhesion family G-protein coupled receptor in endothelial cells. *FEBS Letters* 569, 149-155.

2) During my postdoctoral training the PYRIN domain (PYD) was discovered and we were one of several groups cataloging PYD and CARD proteins in mice and humans and demonstrated significant species differences in the repertoire of these genes (*Genome Res.* 2003, 13, p1376). I demonstrated that ASC has an innate immune function **[a]** and performed one of the three studies that demonstrated that ASC is the activating adaptor for caspase-1 **[b]**. I further discovered and characterized several CARD proteins in regulating inflammatory responses through regulating caspase-1, including the discovery of COP on which in part this application is based **[c]** and NF-κB activation **[d]**.

[a] **Stehlik, C**, Fiorentino, L, Dorfleutner,A, Bruey, JM, Ariza, EM, Sagara, J, Reed, JC (2002) The PAAD/PYRIN-family protein ASC is a dual regulator of a conserved step in NF-κB activation pathways. *J. Exp. Med.* 196,1605-1615, PMC2196065.

[b] **Stehlik, C**, Lee, SH, Dorfleutner A, Stassinopoulos, A, Sagara, J, Reed, JC (2003) Apoptosis-associated speck-like protein containing a caspase recruitment domain is a Regulator of pro-Caspase-1 Activation, *J. Immunol.* 171, 6154-63.

[c] Lee SH, **Stehlik C**, Reed JC (2001) COP, a CARD-containing protein and inhibitor of caspase-1 activation processing. *J. Biol. Chem.* 276, 34495-34500.

[d] **Stehlik, C**, Hayashi, H, Pio, F, Godzik, A, Reed, JC (2003) CARD6, a CARD containing inhibitor of Nod1 and Cardiak induced NF-κB activation. *J. Biol. Chem.* 278, 31941-31949.

3) A main research emphasis in my laboratory is on basic mechanisms of innate immune sensing by cytosolic pattern recognition receptors of the Nod-like receptor (NLR) and AIM2-like receptor (ALR) families. During my postdoc, I discovered NLRC4, NLRP2 and NLRP4 (*Genomics* 2001, 75, p77; *J. Biol. Chem.* 279, p51897; *J. Biol. Chem.* 277, p35333), We recently discovered NLRP7 as an inflammasome activating PRR sensing bacterial lipopeptides, which cooperates with TLR2 in human macrophages during bacterial infection **[a, b]**, discovered a regulatory mechanism for the LPS-induced non-canonical inflammasome **[c]**, and discovered a key homeostatic role of AIM2 in the gut, where it regulates antimicrobial peptide expression through an IL-18BP/IL-22/STAT3 pathway to prevent dysbiosis and susceptibility to colitis **[d]**.

[a] Khare, S, Dorfleutner, A, Bryan, NB, L., Yun, C, Radian, AD, de Almeida, L, Rojanasakul, Y, **Stehlik, C** (2012) An NLRP7-containing inflammasome mediates recognition of microbial lipopeptides in macrophages. *Immunity*, 36, 464-476. PMC3315380. *Highlighted in Nature Reviews Immunology 12, 3, Nature Immunology 13, p358 and by The Faculty of 1000.*

[b] Radian, AD, Khare, S, Dorfleutner, D*****, **Stehlik, C*** (2015) ATP binding by NLRP7 is required for inflammasome activation in response to bacterial lipopeptides, *Mol. Immunol.* 67, 294-302. PMC4565763. *co-corresponding author

[c] Chu, HL, Indramohan, M, Ratsimandresey, RA, Gangopadhyay, A, Morris, EP, Monack, DM, Dorfleutner, A*****, **Stehlik, C*** (2017) The oxidized phospholipid oxPAPC protects from septic shock by targeting non-canonical inflammasome in macrophages, *Nature Communications* 9, 996, PMC5843631. *co-corresponding author

[d] Ratsimandresey, R, Indramohan, M, Dorfleutner, A*****, **Stehlik, C*** (2016) The AIM2 inflammasome is a central regulator of intestinal homeostasis through the IL-18/IL-22/STAT3 pathway. *Cellular & Molecular Immunology,* 14, 127-142. PMC5214942. *co-corresponding author *Top 10 highly accessed article in CMI.*

4) The molecular mechanisms that regulate inflammasomes are still poorly understood. We were the first to describe that endogenous inflammasome components aggregate in then cytosol in macrophages and discovered that ASC is a largely nuclear protein in resting macrophages, which requires inducible redistribution to the cytosol to assemble inflammasomes, which has now been validated and expanded on by several other labs **[a]**. We furthermore described that differential splicing of the inflammasome adaptor ASC regulates inflammasome activation by producing proteins lacking key protein interaction domains and that these splice forms are produced as late response factors to contribute to the resolution of inflammasome responses **[b]**. We also demonstrated bacterial-induced inflammasome responses upon *Pseudomonas* **[c]** *Vibrio* **[d]** and *Salmonella* infection and non-canonical inflammasome regulation **[3d]**, as well as in response to *Listeria* and *Staphylococcus* **[3a,b]** and DNA viruses **[5d]**.

[a] Bryan, NB, Dorfleutner, A, Rojanasakul, Y, **Stehlik, C** (2009) Activation of inflammasomes requires intracellular redistribution of the apoptotic speck-like protein containing a caspase recruitment domain. *J. Immunol.*, 182, 3173-3182, PMC2652671. *Featured "in this issue".*

[b] Bryan, NB, Dorfleutner, A, Kramer, SJ, Yun, C, Rojanasakul, Y, **Stehlik, C** (2010) Differential splicing of the apoptosis-associated speck like protein containing a caspase recruitment domain (ASC) regulates inflammasomes. *J. Inflammation,* 7, 23, PMC2887861. *Highly Accessed*

[c] Kung, VL, Khare, S, **Stehlik, C,** Bacon, E, Hughes, AJ, Hauser, AR (2012) An *rhs* gene of *Pseudomonas aeruginosa* encodes a virulence protein that activates the inflammasome, *Proc. Nat. Acad. Sci. USA,* 109, 1275-1280, PMC3268321. *Highlighted in Nature (2012),* 481, 240.

[d] Queen, J, Agarwal, S, Dolores, JS, **Stehlik, S,** Satchell, KJF (2015) Mechanisms of inflammasome activation by *Vibrio cholerae* secreted toxins varies with strain biotype. *Infect. Immun*., 83, 2496-506. PMC4432742

5) We discovered a key inflammasome regulatory mechanism, which is mediated by PYRIN domain-only proteins (POPs), which act as small endogenous inhibitors and are encoded in humans, but are lacking from mice. We discovered all known full members and established the POP family (*J. Immunol.* 2007, 179, p7993), including poxviral POPs (*Virus Genes*, 2007, 35, p685), POP1 (*Biochem. J.* 2003, 372, p101) **[a]**, POP2 **[b,c]** and POP3 **[d]**, and demonstrated specificity of POPs for a particular inflammasome **[d]** and that POPs are deregulated in inflammatory disease patients **[a]** and that therefore POP-derived peptides have therapeutic significance **[a,c]**.

[a] de Almeida, L, Khare, S, Misharin, AV, Patel, R, Wallin, M, Perlman, HR, Greaves, DR, Hoffman, HM, Dorfleutner, A*, **Stehlik, C*** (2015) The PYRIN domain-only protein POP1 inhibits inflammasome assembly and ameliorates inflammatory disease. *Immunity,* 43, 264-276. PMC4666005. *co-corresponding author, *Highlighted with a Commentary by Shimada K Timothy R Crother TR, and Arditi, M (2015) POPsicle for Fever! Cooling Down the Inflammasome. Immunity, 43, 213-215.*

[b] Dorfleutner, A, Bryan, NB, Talbott, SJ, Fynya, KN, Rellick, SL, Reed, JC, Shi, X, Royanasakul, Y, Flynn, DC, **Stehlik, C** (2007) Cellular PYRIN domain-only protein (cPOP) 2 is a novel regulator of

inflammasome activation. *Infect. Immun.*, 75, 1484-1492, PMC1828547. *Featured as a Spotlight article in Infection and Immunity.*

[c] Ratsimandresey, R, Chu, HL, Indramohan, M, Gangopadhyay, A, Dorfleutner, A*, and **Stehlik, C*** (2017) The PYRIN domain-only protein POP2 regulates inflammasome priming and activation. *Nature Communications*, 8, 15556. PMC5465353. *co-corresponding author

[d] Khare, S, Ratsimandresey, R, de Almeida, L, Cuda, CM, Rellick, SL, Misharin, AV, Wallin, MC, Gangopadhyay, A, Forte, E, Gottwein, E, Perlman, H, Reed, JC, Greaves, DR, Dorfleutner, A*, **Stehlik, C*** (2014) The PYRIN domain-only protein POP3 inhibits AIM2-like receptor inflammasomes and regulates responses to DNA virus infections. *Nature Immunology,* 15, 343-345**,** PMC4123781. *co-corresponding author*, Selected as the cover article and highlighted with a News and Views Commentary by Krishnaswamy JK, Liu D and Eisenbarth SC (2014) POP goes the inflammasome. Nat. Immunol., 15, 311-313.*

Complete List of Published Work: http://www.ncbi.nlm.nih.gov/pubmed/?term=stehlik%3Bc

D. Additional Information: Research Support and/or Scholastic Performance

Ongoing Research Support

R01 AI134030 Stehlik, Dorfleutner (MPI) 07/01/18 - 06/30/23
A novel essential inflammasome component propagating inflammatory responses.
The major goal of this study is to elucidate posttranslational regulation of inflammasome activation and response propagation.
Role: MPI

R01 AI120625 Stehlik, Dorfleutner (MPI) 12/01/15-11/30/20
Molecular regulation of systemic inflammation
The major goal of this study is to dissect mechanisms regulating canonical and non-canonical inflammasomes by POP1.

Role: MPI

R01 AI099009 Stehlik (PI) 03/11/13-02/28/23
Regulation of cytosolic pattern recognition receptor signaling in macrophages
The major goal of this study is to delineate regulation of PRR signaling in inflammatory disease by POPs.
Role: PI

R01 AR064349 Stehlik (PI) 04/01/13-12/31/19
A regulatory checkpoint in the pathogenesis of inflammatory arthritis
The major goal of this study is to determine the function of POP1 and inflammasomes in macrophages during inflammatory arthritis.
Role: PI

R01 AI140702 Dorfleutner, Stehlik (MPI) 07/01/18-06/30/23
CARD-only protein regulation of cytosolic Pattern Recognition Receptor signaling
The major goal of this study is to dissect the role of COPs in key innate immune signaling pathways in MΦ.
Role: MPI

Recently Completed Support

P01 HL071643 Ridge (Project 2 PI) 09/01/15-08/31/20
Role of Role of Intermediate Filaments in Acute Lung Injury
The major goal of this study is to elucidate the role of intermediate filaments in influenza virus-induced inflammasome activation and acute lung injury.
Role: Co-I

R21 AI120618 Stehlik, Dorfleutner (MPI) 06/15/15-05/31/17
A novel mouse model to monitor inflammasome activation in vivo
The major goal of this study is to observe inflammasome activity in the pathology of Cryopyrinopathies (CAPS) and colitis in vivo, utilizing a newly developed inflammasome/caspase-1 reporter mouse model.
Role: MPI