

Endogenous Activators of Inflammation in Insects and Mammals

Bruce Beutler and Jules A. Hoffmann

2007 Balzan Prize for Innate Immunity

Balzan GPC Adviser: Nicole Le Douarin

Researchers: Carrie Arnold, Michael Berger, Amanda Blasius, Philippe Krebs, Oren Milstein, Lei Sun, Sungyong Won (Beutler laboratory); Hidehiro Fukuyama, Anne Kaukinen, Akira Tajima-Goto (Hoffmann laboratory)

Affiliated Institutions: Centre International de Recherche aux Frontières de la Chimie, Strasbourg; The Scripps Research Institute, La Jolla CA

Period: 2008-2012

Bruce Beutler is Professor and Director of the Center for the Genetics of Host Defense, University of Texas Southwestern Medical Center, in Dallas, TX, USA. Jules A. Hoffmann is Distinguished Class Research Director (Emeritus) at the Centre National de la Recherche Scientifique of the Institute of Molecular and Cellular Biology in Strasbourg. The second half of the Balzan Prize to Bruce Beutler and Jules Hoffmann encouraged joint efforts regarding the establishment of a model of inflammation in insects and mammals. The parallel study on inflammation in the absence of germs in the fruit fly (*Drosophila*) and in mice could lead to the future discovery of the causes by which, in humans, antibodies of endogenous origin are also activated in the absence of the pathogenic germs they are supposed to fight, thus producing autoimmune diseases. The two Prizewinners hired young researchers and supervised research work in their respective laboratories, which led to a comparative analysis of the IMD (fly) and TNF/TLR (mouse) proinflammatory signalling pathways in infection and development.

At the Scripps Research Institute in La Jolla, CA (where Dr. Beutler previously worked), Dr. Michael Berger screened peptidomimetic libraries for activators of TLR signalling. These studies, designed to identify molecules that could cause unconventional activation of TLR signalling, were performed as a collaboration with

the laboratory of Professor Dale Boger at the Scripps Research Institute. Dr. Oren Milstein searched for immune activating functions of peptides that do not exist in the mouse proteome. Dr. Philippe Krebs studied mutations that cause inflammatory disease and their attenuation by mutations that disrupt TLR signalling. Particularly significant was his demonstration that signalling via TLRs drives the lethal inflammatory disorder observed in mice with deficiency of the inositol polyphosphate 5 phosphatase, SHIP-1. Drs. Sungyong Won and Lei Sun worked jointly to develop a technique for cloning mice from fibroblasts, with the goal of screening these cells en masse for ex vivo phenotypes (including spontaneous inflammatory phenotypes) before regenerating mice from them and positionally cloning the causative mutations. Dr. Carrie Arnold initiated a screen for defects in the adaptive immune response, and succeeded in identifying eleven mutations to date. Dr. Amanda Blasius identified a key molecule for the responses of plasmacytoid dendritic cells to nucleic acids. More recently, in Dallas TX, Dr. Lijing Su co-crystallized certain chemicals and endogenous molecules that trigger TLR responses together with the TLRs to which they bind.

In Strasbourg, Dr. Hidehiro Fukuyama pursued a biochemical strategy to identify proteins that interact with components of the stands for immune-deficiency pathway (IMD, homologous to mammalian TNF) in *Drosophila* to limit inflammation caused by endogenous stimuli. Dr. Anne Kaukinen made a functional analysis of some of the proteins isolated by Dr. Fukuyama, and namely addressed their potential roles in activating antimicrobial peptide gene expression following stimulation by a bacterial pathogen. Exciting new data point to a significant role of the IMD signalling pathway in the defence of flies against several viral pathogens. The Balzan funds for Professor Hoffmann's group concentrated on developing this new line of research. Professor Hoffmann gave a lecture entitled *Gene Expression and Signalling in the Immune System* at the sixth Cold Spring Harbor meeting in April 2012. He was also keynote speaker at the following conferences:

- 22nd ECCMID-ESCMID, European Society of Clinical Microbiology and Infectious Diseases. Keynote Lecture. Conference on "Phylogenetic Perspectives in Innate Immunity." London, UK. April 2012.
- 26th Conference of European Comparative Endocrinologists. Keynote Lecture. "Phylogenetic Perspectives of Innate Immunity." Zurich, Switzerland, August 2012.

Publications

- Fukuyama, H., Ndiaye, S., Hoffmann, J., Rossier, J., Liuu, S., Vinh, J., Verdier, Y. 2012. On-bead tryptic proteolysis: An attractive procedure for LC-MS/MS analysis of the *Drosophila caspase* protein complex during immune response against bacteria. *Journal of Proteomics*. DOI:10.1016/j.jprot.2012.03.003.
- Liu, X., Sano, T., Guan, Y., Nagata, S., Hoffmann, J.A., Fukuyama, H. 2012. *Drosophila* EYA Regulates the Immune Response against DNA through an Evolutionarily Conserved Threonine Phosphatase Motif. *Journal PLoS One*. 7(8): e42725. DOI:10.1371/journal.pone.0042725 (15.08.2012).
- Kemp, C., Mueller, S., Goto, A., Barbier, V., Paro, S., Bonnay, F., Dostert, C., Troxler, L., Hetru, C., Meignin, C., Pfeffer, S., Hoffmann, J.A., Imler, J.L. 2013. Broad RNA interference-mediated antiviral immunity and virus-specific inducible responses in *Drosophila*. *The Journal of Immunology*. Vol 2, 650-658.
- Fukuyama, H., Verdier, Y., Guan, Y., Makino-Okamura, C., Shilova, V., Liu, X., Maksoud, E., Matsubayashi, J., Hhaddad, I., Spirohn, K., Ono, K., Hetru, C., Rossier, J., Ideker, T., Boutros, M., Vinh, J., Hoffmann, J.A. 2013. Landscape of protein-protein interactions in *Drosophila* immune deficiency signaling during bacterial challenge. *Proceedings of the National Academy of Sciences USA*. Vol 26, 10717-10722.
- Su, L., Wang, Y., Wang, J., Mifune, Y., Morin, M.D., Jones, B.T., Moresco, E.M.Y., Boger, D.L., Beutler, B., Zhang, H. 2019. Structural Basis of TLR2/TLR1 Activation by the Synthetic Agonist Diprovocim. *J Med Chem*. 2019 Mar 28;62(6):2938-2949. doi: 10.1021/acs.jmedchem.8b01583. Epub 2019 Mar 13.
- Wang, Y., Su, L., Morin, M.D., Jones, B.T., Whitby, L.R., Surakattula, M.M., Huang, H., Shi, H., Choi, J.H., Wang, K.W., Moresco, E.M., Berger, M., Zhan, X., Zhang, H., Boger, D.L., Beutler, B. 2016. TLR4/MD-2 activation by a synthetic agonist with no similarity to LPS. *Proc Natl Acad Sci USA*. 2016 Feb 16;113(7):E884-93. doi: 10.1073/pnas.1525639113. Epub 2016 Feb 1.