

A scenic view of a turquoise lake with mountains in the background and a small island with a tree and a boat in the foreground. The sky is blue with white clouds. The text "Summer School 2012 Bönigen" is overlaid on the image.

Summer School 2012

Bönigen





tion, Immunomodulation, Inspiration

— 11th International Summer School

Organisation:
Prof. Hans-Uwe Simon, MD, PhD

12-14, 2012
Hotel La Terrasse, CH – 3806 Bönigen

Marafa (Bern)
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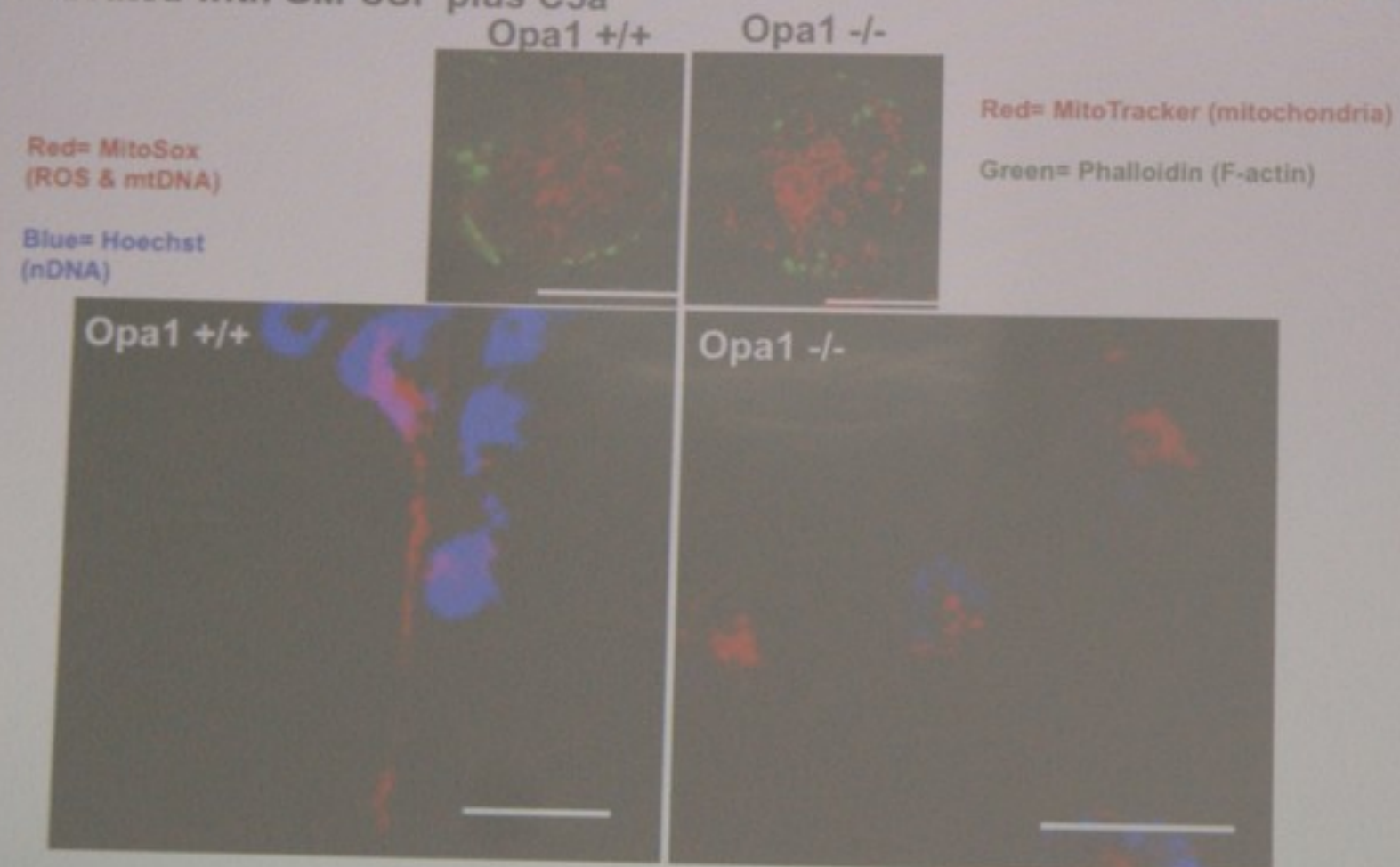


mtDNA release also requires mitochondria fusion protein; Opa1

5 days differentiated HoxB8 mature mouse neutrophils

Activated with GM-CSF plus C5a

See Poorya' poster



IgE-dependent and independent activation induce
basophils mitochondrial DNA release

Mahbubul Morshed

Institute of Pharmacology

University of Bern

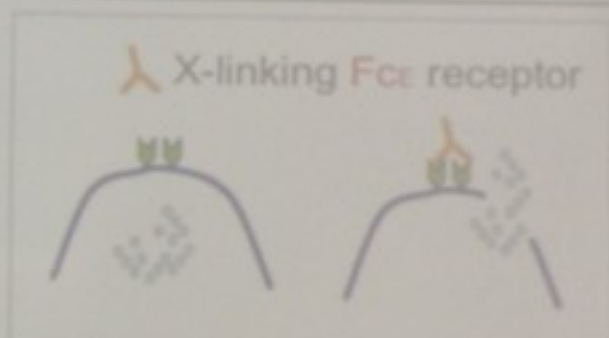
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Summer School 12.08.2012, Bönigen



Fc Receptors

1. $Fc\gamma$ for IgG binding
2. $Fc\alpha$ for IgA, IgM binding
3. $Fc\epsilon$ for IgE binding



- a. High affinity $Fc\epsilon R1$ – expressed in basophils, mast cells, Langerhans cells.
- b. Low affinity $Fc\epsilon R2$ – expressed in B cells, Langerhans cells.

Monoclonal $Fc\epsilon R1\alpha$ antibody does not inhibit the binding of IgE. It induces basophil activation followed by histamine release.

Hakimi, J. et al. The alpha subunit of the human IgE receptor ($Fc\epsilon R1$) is sufficient for high affinity IgE binding. *J. Biol. Chem.* (1990).



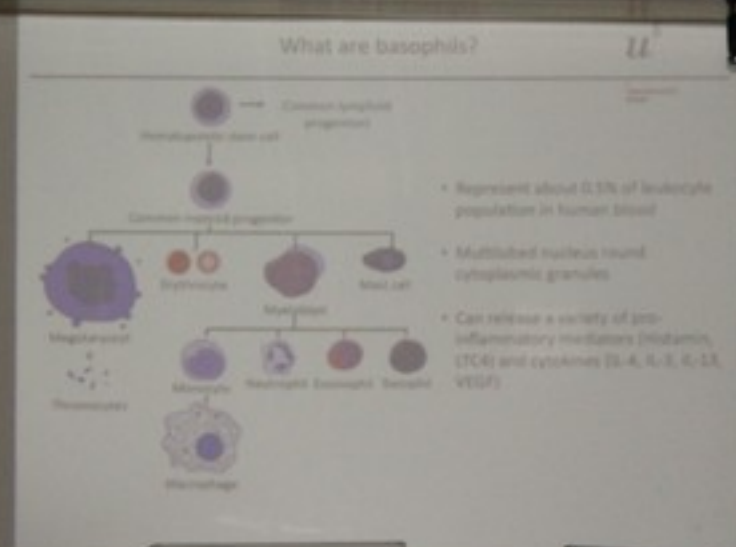




Research Interests

- Cell death regulation by BCL-2 and IAP proteins
- Crosstalk from death receptors to mitochondria
- Non-apoptotic roles of XIAP (NODosome, Inflammasome)
- in vitro models for basophils and mast cells



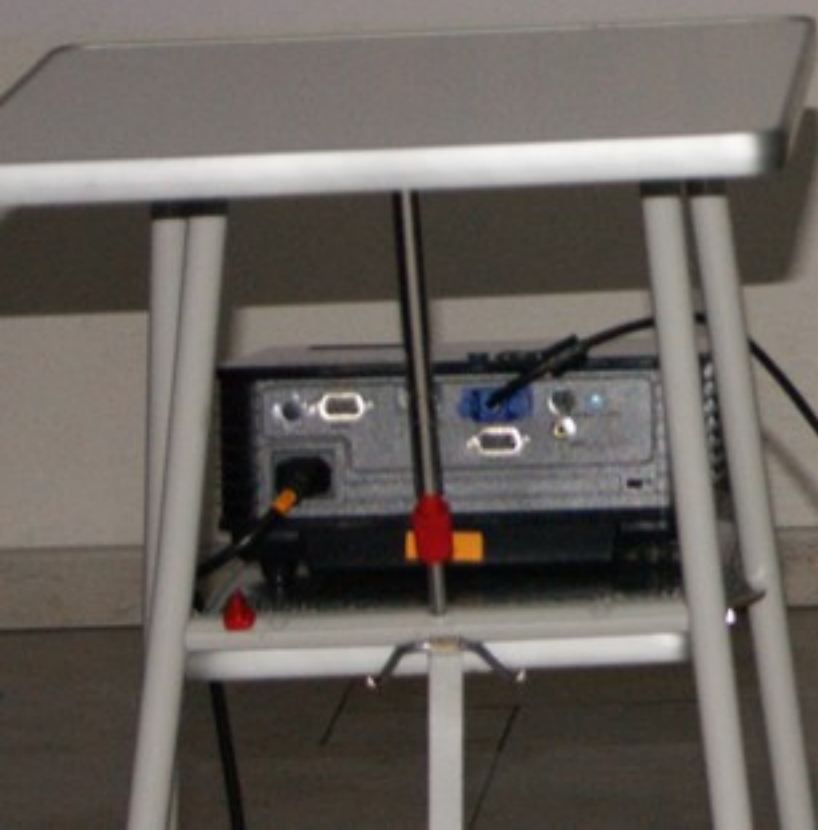


August 12, 2012

A Role for Siglecs in Tumor Immunology?

Stephan von Gunten, MD, PhD, MME

Institute of Pharmacology, University of Bern
www.pki.unibe.ch







Restaurant

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Thanks to....

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Prof. Dr. Verena Niggli
PD Dr. Jens Stein

Supervisor
Mentor
Co-referee

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S1P1-receptor internalization experiment

TKI

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for
EAE-experiments

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for
T-cells

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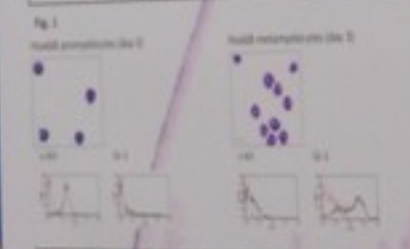
Neutrophil differentiation is regulated by Atg5

Sela Rubman¹, Charaf Benarafa², Mathias Baumann², Kerstin Simon¹
¹Institute of Pharmacology and ²Theodor Kocher Institute

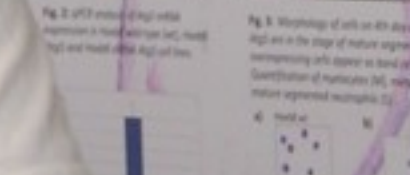
Autophagy is an essential self-degradation pathway, during which organelles and proteins are sequestered into autophagosomes, and delivered to lysosomes for subsequent degradation. Autophagy is therefore an essential process for cellular homeostasis, and is also involved in many other cellular processes, such as cell differentiation. Autophagy is also involved in the differentiation of neutrophils, which are essential for the immune response. However, the role of autophagy in neutrophil differentiation and function has up to now been poorly described.

Using immortalized neutrophil progenitor cell lines and myelocyte-specific differentiation,

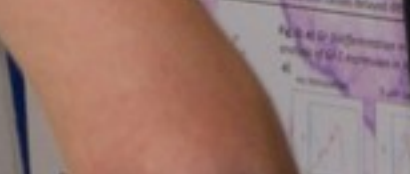
we have established a model to study neutrophil differentiation. In this model, we have shown that autophagy is essential for the differentiation of neutrophils, and that the autophagy protein Atg5 is a key regulator of this process.



Atg5 expression regulates differentiation in Hoxb8 cell lines. Modulation of Atg5 expression in Hoxb8 cells (overexpression and knockdown) affects the differentiation of neutrophils. Atg5 overexpression leads to increased differentiation, while Atg5 knockdown leads to decreased differentiation.



Autophagy in Hoxb8 cell line. Hoxb8 cells are a model for studying neutrophil differentiation. In this model, we have shown that autophagy is essential for the differentiation of neutrophils, and that the autophagy protein Atg5 is a key regulator of this process.



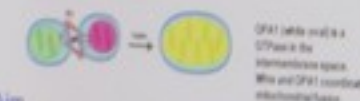
Physiological functions of Opa1, an important mitochondrial fusion protein in neutrophil viability

Poorya Amini¹, Darko Stojkov¹, Kevin Oberson, Mahbubul Morshed¹, Christina Gerth-Kahlert², André Schaller³, Maria Eugenia Soriano Garcia-Cuerva², Luca Scorrano², Shida Yousefi¹, and Hans-Uwe Simon¹

¹Institute of Pharmacology, University of Bern and ²Dubecco-Telethon Institute, Venetian Institute of Molecular Medicine (VIMM), Padua, Italy
³Eye Clinic, University Hospital Zurich, ⁴Division of Human Genetics, University of Bern Children hospital

Background

In recent years, the dynamic nature of mitochondria has been discovered to be critical for their function. OPA1, a GTPase associated with the mitochondrial inner membrane, mediates inner-membrane fusion of mitochondria. Autosomal dominant optic atrophy (ADOA) is the most common inherited optic neuropathy that affects the optic nerves, causing reduced visual acuity and blindness beginning in childhood. Dominant optic atrophy is associated with mutation of the OPA1 gene. Mitochondrial fusion enables content mixing within a mitochondrial population, thereby preventing permanent loss of essential components. Cells with reduced mitochondrial fusion show a subpopulation of mitochondria that lack mtDNA nucleoids. Such mtDNA defects lead to respiration-deficient mitochondria, and their accumulation in cells leads to impaired cellular functions and ultimately diseases, such as neurodegeneration.



Aim

We investigated role of Opa1 in spontaneous as well as Fas-induced cell death in neutrophils. Furthermore, the role of Opa1 in dynamic nature and morphology of neutrophil mitochondria.

Methods

We generated mice lacking Opa1 gene specifically in myeloid population, by crossbreeding Opa1^{fl/y} with Lys2^{Cre} mice. These mice have Opa1 knockout restricted to myeloid lineage (Opa1^{fl/Cre}). In addition, we used conditional Hoxb8-expression to generate mouse pre-neutrophil cell line that lacks Opa1 and upon withdrawal of Tamoxifen within 5-6 days differentiates to fully mature neutrophils (Hoxb8 Opa1^{fl/Cre}). Peripheral centrifugation and subsequent lysis of red blood cells. Cell death was assessed by flow cytometry. To determine whether cell death was apoptosis, annexin V staining was analyzed. Caspase-3 activation was assessed by immunoblotting and enzymatic assay. For mitochondrial morphology, cells were seeded on 488 and Hoechst. Slides were analyzed by confocal laser scanning microscopy. To measure reactive oxygen species (ROS) cells were primed with GM-CSF and by stimulation and subsequently analyzed by flow cytometry.

Result

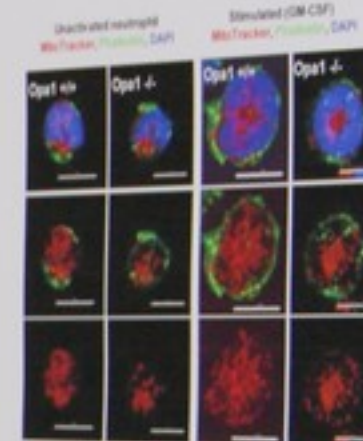
Changes in mitochondria morphology in ADOA patient neutrophil, lacking Opa1 fusion gene



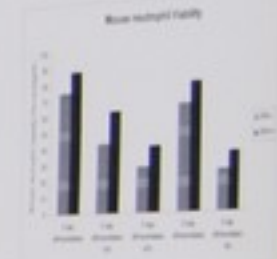
Conclusion

These preliminary results demonstrate that OPA1 plays an important role in mitochondrial fusion and neutrophil viability.

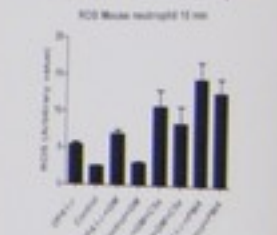
Decreasing of mitochondria fusion in absent of OPA1 in mature mouse Hoxb8 neutrophil



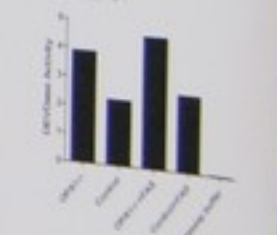
Mouse Hoxb8 OPA1-/- neutrophils are less viable than OPA1+/+



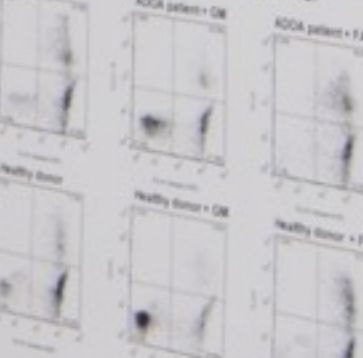
Increased ROS activity in mouse OPA1-/- neutrophil



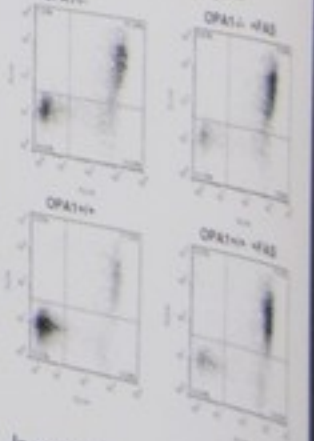
Caspase-3 activity is higher in mouse OPA1-/- neutrophil than OPA1+/+



Increased PS redistribution in ADOA patient neutrophil (Annexin V staining)



Increased PS redistribution in mouse OPA1-/- neutrophil



Increased caspase-3 cleavage in ADOA patient neutrophil in comparison to healthy donor



Increased caspase-3 cleavage in mouse neutrophil OPA1-/- in comparison to OPA1+/+



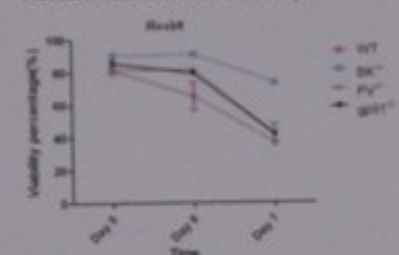
CROSS TALK BETWEEN Ca^{2+} & ROS UPON PHYSIOLOGICAL ACTIVATION OF NEUTROPHILS

Yara Amini¹, Mahbubul Morshed¹, Sasa Rozman¹, Shida Yousefi¹ and Hans-Uwe Simon¹
¹Institute of Pharmacology, University of Bern

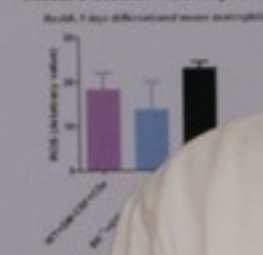
Pathogens, neutrophils possess a potent machinery to produce reactive oxygen species (ROS), the adenine dinucleotide phosphate (NADPH) oxidase. ROS is crucial in the execution of most antimicrobial mechanisms. Variations in $[\text{Ca}^{2+}]$ have been linked with neutrophils production of reactive oxygen species. An increased production of reactive oxygen species and an enhanced calcium entry in neutrophils stimulated with C5a suggests that ROS activity could serve as an agonist to reinforce calcium signaling.

Results

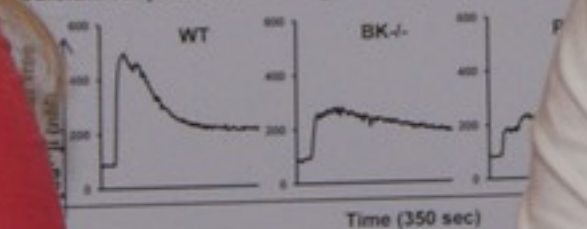
Viability of genetically modified Hoxb8 mature mouse neutrophils



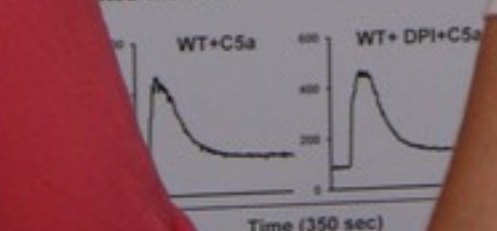
ROS production by Hoxb8 mature mouse neutrophils



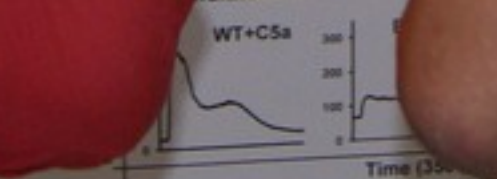
Calcium response to C5a in genetically modified Hoxb8



Calcium response in Hoxb8 mature mouse neutrophils pre-treated with DPI and stimulated with C5a



Calcium response in human neutrophils stimulated with C5a



Significant decrease of superoxide anion production was observed after treatment with DPI before stimulating with C5a. BAPTA also caused a significant decrease of $[\text{Ca}^{2+}]$ concentration. Using estrogen-regulated Hoxb8 mouse neutrophils, a high level of ROS activity leads to sustained high level of $[\text{Ca}^{2+}]$ due to Ca^{2+} entry.

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The role of proteinase 3 (PR3) in neutrophil death

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Background

Apoptosis is the most common cell death of neutrophils, but other types of death may occur under certain conditions. Recently, our group reported a novel form of programmed necrotic cell death induced by GM-CSF and other inflammatory cytokines upon ligation of CD44. Proteinase 3 (PR3), a serine protease stored in the azurophilic granules of neutrophils, has been identified as a major c-ANCA (cytoplasmic anti-neutrophil cytoplasmic antibodies) antigen in Wegener's Granulomatosis (WG). Elevated expression levels of PR3 have been observed in WG and some other chronic inflammatory diseases.

To test the hypothesis that adhesion molecules (such as CD44) trigger PR3-mediated neutrophil death.

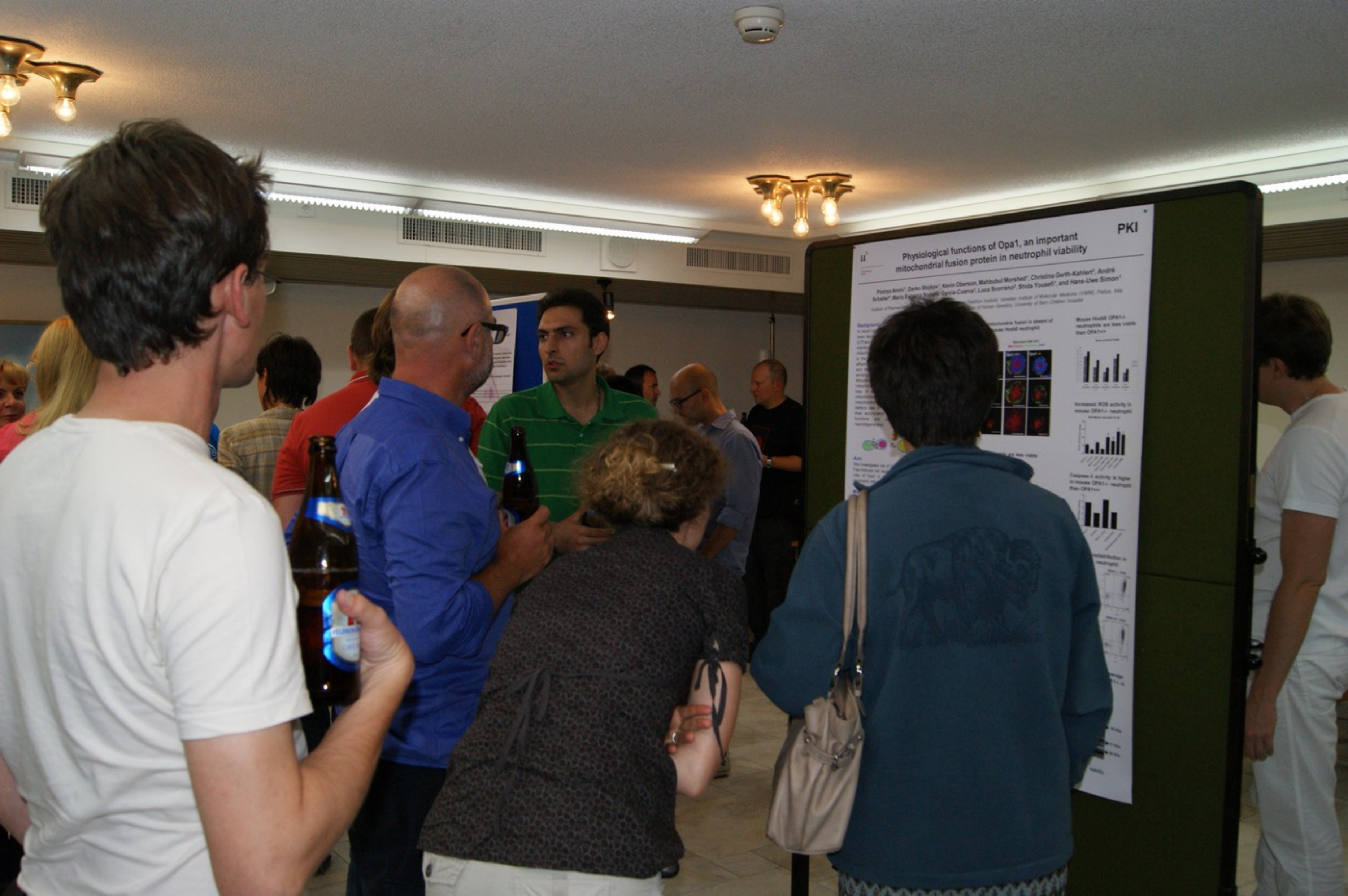
Results



Measurements

Cell death is assessed by uptake of Ethidium bromide, by flow cytometry analysis. For ROS production, neutrophils are stimulated as described and incubated at 37°C for 30 min. ROS are measured by flow cytometry.





Physiological functions of Opa1, an important mitochondrial fusion protein in neutrophil viability

PKI

Poorna Anand¹, Darko Stojkovic¹, Kevin O'Brien¹, Mahbubul Morshed¹, Christina Gerth-Kahlert², André Schaller¹, Maria Eugenia Soriano Garcia-Cuervo¹, Luca Scornano², Shida Yousefi¹, and Hans-Uwe Simon¹

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