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Assays for chemotaxis and chemokine inhibitors

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Abstract

Chemokines play a crucial role in immune response and the development, homeostasis and regulation of the immune system. They regulate the migration of various types of leukocytes through interaction with their receptors [1]. The aim of this work is to inhibit interleukin-8 (IL-8, CXCL8) and eotaxin (CCL11) to prevent leukocyte migration in inflammatory diseases. The migration of leukocytes can be determined with various activity assays and microscopic methods. The first step in assay development is to find out which concentration of the chemokines has the strongest effect. Once assay conditions have been optimized potential inhibitors can be tested. These inhibitors can be peptides with a similar amino acid sequence like the CXCL8 and CCL11 receptors but also natural products like naringenin. Before the migration of leukocytes can be studied the cells have to be differentiated into neutrophil and eosinophil granulocytes. To show after how many days differentiation was complete Western Blot analysis was performed.

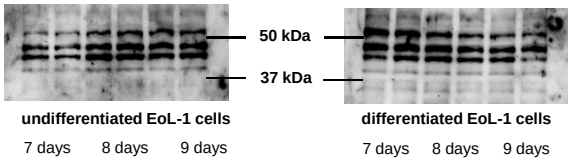
Cell culture models for granulocyte based assays

The human eosinophilic leukemia cell line EoL-1 can be differentiated into eosinophils with apicidin, and the human myeloid cell line HL-60 can be differentiated into neutrophils with DMSO and granulocyte colony stimulating factor (G-CSF).

Both cell lines were cultivated in RPMI medium supplemented with 15 % fetal bovine serum at 37°C. The EoL-1 cells were induced to differentiate with 100 nM of apicidin at different time points [2]. The HL-60 cells were treated with 1,25 % dimethylsulfoxid and 25 ng/ml G-CSF for 6 days [3].

Test for differentiation markers

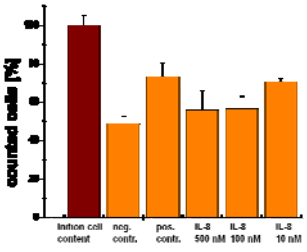
- EoL-1 cells were differentiated 7, 8, and 9 days with 100 nM apicidin.
- Cell lysates were tested for eotaxin receptor CCR3 by Western Blot
- Molecular weight of CCR3: 41 kDa



➞ **CCR3 was expressed in undifferentiated and differentiated cells.**

Migration Assay

- Idea: Differentiated HL-60 cells migrate upon interaction with interleukin-8 in direction of the concentration gradient.
- The assay was established with a transwell system
- Procedure: Differentiated HL-60 cells were suspended medium and a total of 1x 10^6 cells were added into the upper well of the transwell inserts (membrane with 3 µm pores). The lower wells were filled with medium containing different concentrations of IL-8. After an incubation time of 30 min the cells in the upper and lower wells were counted with the Casy cell counter and the number of migrated cells calculated.

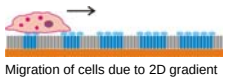


IL-8 dependent chemotaxis of differentiated HL-60 cells. Initial cell number is expressed as 100 %, and IL-8 chemotaxis is expressed as a percentage of the initial cell number. Positive control: 100 nM fMLP; Negative control: chemotaxis medium

➞ **Optimum chemotaxis response at 10 nM IL-8 after 30 min incubation**

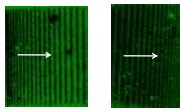
Cell migration of microstructured surfaces

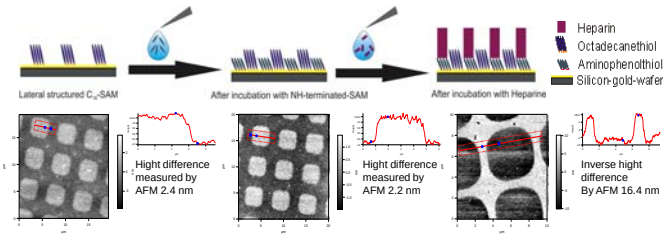
Proteins printed on surfaces in stripes of increasing thickness or with decreasing spacing are preceived as protein gradients by several cell types [4]. We are testing different methods to produce interleukin-8 patterns to study the response of neutrophils to these patterns.


Migration of cells due to 2D gradient

Microcontact printing (µCP)

- Idea: Microstructured self-assembled monolayers (SAM) of alkane- or aminothiols on gold surfaces
- Adsorption of chemokines to alkane chains
- Or: Adsorption of heparin to aminophenolthiol and of chemokine to heparin.


Gradient patterns of FITC by µCP investigated by fluorescence microscopy (FITC-filter)



- Procedure: A PDMS stamp with a lateral pattern was loaded with a solution of octadecanethiol. The thiol pattern was printed on a gold coated silicon wafer. The silicon wafer was incubated with aminophenolthiol and then with heparin for 1 hour. Hight profiles of the surface were measured after every incubation step to control surface topography.

Conclusions and Outlook

- CCR3 can´t be used as a differentiation marker for EoL-1 cells.
- Proteoglycan2, Peroxidasin and CD11b will be tested as potential differentiation marker for EoL-1 cells.
- IL-8 shows the strongest effect on the migration of HL-60 at a concentration of 10 nM.
- Potential inhibitors for IL-8 will be tested in the migration assay.
- Recombinant IL-8 will be analyzed in several activity assays.
- AFM data shows the selective binding of heparin to amino-terminated thiols.
- The binding of IL-8 to heparin will be also investigated by AFM.
- The migration of leukocytes on microstructured surfaces will be characterized.

References

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[4] A. C. von Philipsborn, *Development* **2006**, *133*, 2487-2495.

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