Display of a Full Length IgG Antibody on the Surface of *Escherichia coli*: Towards the Screening of an Antibody Library

Dilkaute, C.; Weckenbrock, W.; Jose J.

Institute of Pharmaceutical and Medicinal Chemistry, PharmaCampus, Westfalian Wilhelms-University, Corrensstraße 48, 48149 Münster, Germany

In today's clinical practice, monoclonal antibodies have become a well-established therapy option for a range of indications, such as cancer and autoimmune diseases [1]. To develop various specific antibodies, huge antibody libraries have to be screened. For this purpose phage display has been used with great success in the last 25 years. Nevertheless, this method is associated with some drawbacks as the possible discrimination of the most potent binders during the biopanning process, the incompatibility with flow cytometry or the size limitation of the protein displayed on the surface [2]. To circumvent these disadvantages, we developed a screening tool using *E. coli* cells presenting a full-length antibody on their surface. The presentation of antibodies and in particular their libraries enables the screening for new variants against pre-given epitopes using flow-cytometry without losing the highly potent binders.

In this work, the autodisplay technique [3,4] was utilized to present a functional full-length antibody on the surface. As a proof of principle, the display of the antibody T84.66 which is directed against carcinoembryonic antigen (CEA) was investigated. Based on this antibody a library was generated. Therefore, restriction sites were introduced in front of and behind the complementarity determining region 3 (CDR3). This enables the exchange of the CDR3 through a randomized fragment. After ligation, this construct was used to transform *E. coli* UT5600 (DE3) via electroporation. The resulting library consists of up to 10^5 clones which can be analysed and sorted via flow cytometry after incubation with a fluorescently labelled target protein. To examine the optimal conditions for the screening, two different autotransporters in combination with two promoters were investigated: the AIDA-1 autotransporter [3] under control of a T7-promoter and the EhaA-autotransporter [4] controlled by an araBAD promoter. Experiments with the T84.66 antibody as a passenger revealed that the EhaA-autotransporter under araBAD control suited better with regard to surface presentation and cell survival after sorting via flow cytometry.

These results indicate that it is possible to generate a full-length antibody library on the surface of *E. coli* which afterwards can be screened with the advantageous high-throughput screening system of flow cytometry. Further investigations should be performed to identify an antibody variant out of the constructed library which binds a pre-given epitope of therapeutical interest.

References:

1. Weiner L. M. et al.: *Nat Rev Immunol* **2010**, 10(5): 317-327
2. Levin A. M., Weiss G.A.: *Mol. BioSyst* **2006**, 2: 49-57
3. Jose J., Meyer T.F.: *Microbiol. Mol. Biol. Rev.,* **2007,** 71(4): 600-619
4. Sichwart S. et al.: *Food Technol. Biotechnol.* **2015**, 53(3): 251-260