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## Targeting the intracellular proteome: Novel therapeutic antibodies that mimic T cell specificity

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The foundations of targeted cancer therapy depend on the identification and targeting of unique, cell surface-expressed tumor-associated proteins, which distinguish tumor cells from healthy cells. The most effective way to target these proteins is by using monoclonal antibodies (mAbs). However, the number of these unique tumor-associated proteins is very limited because only a minority of our proteome is expressed on the surface of cells; the majority of the human proteome is expressed intracellularly. As many disease-associated cell surface proteins are discovered and patented, the number of new, available, unique cancer markers declines. Thus, there is a great need to develop mAbs against intracellular targets. This is AIT's domain of expertise. AIT's innovation stems from two unique and proprietary capabilities: 1) the ability to identify and validate novel intracellularly-derived disease-specific targets, and 2) the ability to generate therapeutic and diagnostic T-Cell receptor Like (TCRL) antibodies against these intracellular-derived targets. The TCRLs then bind specifically to, and kill, the diseased cells. Thus, AIT can transform disease-specific targets that are expressed inside malignant cells into targets that can be recognized on the cell surface by soluble TCRL antibodies. This approach expands the market of novel therapeutic antibodies beyond the limits of currently available antibodies. AIT is also developing a unique strategy to discover new MHC-based targets that can be applied to the isolation and characterization of new TCRL antibodies against a variety of disease-related intracellular targets. This strategy combines basic principles of immune recognition/function with proteomics and computational biology approaches. The large intracellular proteome, which is not accessible for antibody-based recognition, can serve as a huge pool for new target discovery if proteomic strategies and the TCRL technology are combined together. When combined, such a strategy may provide an excellent pipeline for therapeutic and diagnostic antibodies.

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## Isolation of *Balamuthia mandrillaris*- Specific antibodies from a bacteriophage antibody display library

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Balamuthia mandrillaris is an emerging protozoan pathogen that can cause fatal encephalitis in humans and animals. Little is known about the pathogenesis, pathophysiology of Balamuthia amoebic encephalitis and its clinical diagnosis. In this study, we successfully isolated antibody fragments which specifically bind to *B. mandrillaris* using a bacteriophage antibody display library. Using two positive panning rounds, followed by a subtractive pan against *Acanthamoeba* and finally a positive panning round against *B. mandrillaris*, we were able to enrich several clones of phage library expressing single chain Fv antibody fragments (svFv) with binding affinity for *B. mandrillaris*. Individual clones were studied by enzyme-linked immunosorbent assays, as well as immunofluorescence assays and specific binding to *B. mandrillaris*. In conclusion, we were successfully able to isolate antibody fragments specific to *B. mandrillaris* using a phage antibody display library, which may also be useful in the identification as well as differentiation of other species of *B. mandrillaris* for diagnostic applications, and may identify novel surface proteins.

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