

Generation of anti-orthopoxvirus fully human Mabs derived from phage display single-chain antibodies

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Concerns regarding the reemergence of monkeypox as a major human disease in Africa and other countries, and the use of variola virus for bioterrorism have fueled renewed interest in vaccinia virus (VACV) as a vaccine for orthopoxvirus infections. Although VACV is a generally safe vaccine, disseminated, life-threatening infections occur infrequently, especially in individuals with impaired immunity. Such infections can be treated by therapeutic administrations of human VACV immune globulin (VIG). Human monoclonal antibodies offer an obvious alternative to VIG, but human hybridomas are often unstable and/or poor producers, thus making the production of large amounts of the human immunoglobulin very difficult. The use of human polyclonal immunoglobulins or human immune sera is restricted by variable characteristics for the different lots of preparation, low concentration of specific antibodies and existing possibility of contamination by the infection agents. To counter the problems, genetic engineering has developed methods to replace mouse parts in monoclonal antibodies with more human and, presumably, less immunogenic sequences. One possible approach for development of fully human Mabs is selection of variable fragments of human immunoglobulins from combinatorial phage library and then combination them with human IgG constant regions.

To develop fully human Mabs against orthopoxviruses, a new phage-display library of human scFv antibodies was generated from Vh and Vl genes cloned from the peripheral lymphocytes of vaccinia virus immune donors. Populations of peripheral lymphocytes were educated when the titers of anti-VACV antibodies in the serum peaked up to the plateau. The genes encoding the variable heavy and light chain domains were amplified by RT-PCR reaction using the primers specific to the conservative regions of these genes. Vh and Vl genes were randomly combined via the DNA linker whose structure corresponded to Ser(Gly₄Ser)₂AlaArgGlySerGly₄Ser sequence. The scFv genes were cloned into pHEN2 phagemid vector and the library was constructed using the *Escherichia coli* TG1 strain. Library size was calculated to be 3×10^7 members. Library quality was verified by determining the percentage of clones with an insert in the appropriate site for scFv, it was indicated that 20/20 clones contained an scFv-sized insert, and nine scFvs from ten clones could be produced as soluble scFvs.

This library was panned against vaccinia virus and positive clones were selected. Selected scFvs were tested in simultaneous ELISA experiments for binding with vaccinia, cowpox and ectromelia viruses. Most of the tested scFvs reacted with the viruses in equal manner, but several scFvs were found to be species-specific. A standard assay of virus neutralization as the ability of antibodies to inhibit viral plaque formation in cells monolayer was performed with the studied viruses, and several scFvs showed neutralizing activity against the orthopoxviruses. Selected scFvs were then engineered to the whole IgG1 human molecules and produced in HEK293T cells using transient expression. After purification by affine chromatography, ELISA and neutralization assays were repeated. In addition, Western-blot analysis was used to identify the target proteins for the recombinant human IgG.