Abstract
8th meeting of the WHO Advisory Committee on Variola Virus Research, 16-17 November 2006, Geneva, Switzerland

Development of neutralizing human scFv-antibodies against pathogenic orthopoxviruses

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Although vaccinia virus is usually a safe vaccine, sometimes it leads to disseminated, life-threatening infections, especially in immunocompromised individuals. Such infections can be treated by therapeutic administrations of human vaccinia immune globulin (VIG). However, the use of human polyclonal immunoglobulins or human immune sera has its limitations, i.e. the characteristics of this product can vary from batch to batch; low concentrations of specific antibodies, and possible biological risks associated with donor blood use. To solve these problems, genetic engineering has developed approaches to replacing murine parts in monoclonal antibody molecules by the human ones. One of the possible approaches is to select variable fragments of human immunoglobulins from combinatorial phage libraries and then combine them with human IgG constant regions. A key step here is selection of antigen-binding domains from a combinatorial library that possess the desired properties, i.e. are capable of neutralizing the infectivity of orthopoxviruses.

A new phage-display library of human scFv antibodies has been generated from Vh and Vl genes cloned from the peripheral lymphocytes of vaccinia virus immune donors. This library was panned against vaccinia virus and cowpox virus. Enrichment of the library was tested in solid-phase ELISA and PRNT. This data confirmed an increase in anti-orthopoxvirus antibody concentrations and in the titer in PRNT. In addition, an increase in antibodies capable of binding the 33-35kDa orthopoxvirus protein was demonstrated by western-blot following library purification. Then, from a population of antibodies enriched against vaccinia virus, 14 unique mini-antibodies able to neutralize certain orthopoxviruses, including monkeypox virus, cowpox virus, vaccinia virus and ectromelia virus, were selected. Eight of them were specific against the same 33-35 kDa protein of monkeypox, cowpox, vaccinia, and ectromelia viruses.

To identify target protein for the eight antibodies, an E.coli strain producing recombinant protein J3L-β-galactosidase was developed, where J3L is the cowpox analog of vaccinia virus (Copenhagen) protein encoded by ORF H3L. This recombinant protein was detected by sera of vaccinated donors. It turned out that the eight p35-specific neutralizing antibodies also bound recombinant protein J3L. This finding confirms data published earlier that the protein encoded by ORF H3L is one of the major immunodominant orthopoxvirus proteins for human organisms.