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Session 5: Infectious diseases – I

Thursday 9 October 2003. Moderators: Roberto Burioni and Miroslav Gorny

[08.30–09.15]
[Keynote Lecture]
Human monoclonal antibodies as therapeutics in infectious diseases
Alois Lang
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Abstract not received.

[09.15–09.45]
The V3 epitopes on HIV-1 particles are accessible to neutralizing human anti-V3 mAbs
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Anti-V3 antibodies (Abs) are induced by natural HIV-1 infection in all infected individuals as shown by their presence in sera, and can also be elicited experimentally by various candidate vaccines. V3 Abs, both polyclonal and monoclonal (mAbs), have the capacity to neutralize viruses from many clades which use X4 and/or R5 coreceptors. Their neutralizing activity, however, may be limited by antigenic variation of the V3 region, or by lack of V3 exposure on the surface of intact virions. To clarify this issue, accessibility of V3 epitopes on clade B viruses to human anti-V3 mAbs was determined using binding and neutralization assays.

Human anti-V3 mAbs were produced via fusion of heteromyeloma cells with EBV-transformed peripheral blood mononuclear cells (PBMC) derived from HIV-infected individuals. The V3 mAbs were selected with a V3-fusion protein which retains the native conformation of the V3 domain of HIV-1 gp120. A panel of seven human anti-V3 mAbs was tested for neutralizing activity against 13 clade B viruses including one T-cell laboratory adapted strain (X4), five long-term passaged R5 strains, three molecularly cloned X4R5 strains, and four R5 primary isolates. Neutralization was measured in a single-round PBMC assay. Reactivity of mAbs with V3 epitopes was measured in two binding assays: The virus capture assay measures the binding of mAbs to oligomeric structures on the surface of intact virions; the binding of mAbs to detergent-solubilized gp120 identifies the presence of V3 epitopes on the monomeric molecules.

Twelve out of 13 viruses showed significant neutralization by one or more anti-V3 mAbs at 50 μg/ml. Four long-term passaged viruses were neutralized by all V3 mAbs, five viruses were neutralized by 2–6 mAbs, and three strains were neutralized by one mAb. Regression analysis of data revealed that strength of binding of V3 mAbs to intact virions and to soluble gp120 correlates with potency of neutralization ($p < 0.0001$). Similarly, relative affinity of V3 mAbs measured by 50% of maximal binding to intact virions also correlates significantly with neutralization ($p < 0.0001$). These results show that with few exception, the V3 loop is accessible on the native virus envelope and the epitopes of V3 may be shared rather than isolate-specific.

[09.45–10.15]
Human antibodies to agents of biological terrorism
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Due to the purposeful release of anthrax spores in the United States and the follow-up threats of other attacks, biological terrorism is now a reality. We have recently applied our expertise in human combinatorial antibody library technologies towards infectious diseases

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related to threats of biological terrorism, with a focus on passive immunity. Phage display libraries expressing human antibody Fab fragments were created from blood and bone marrow of human donors immunized against potential biological terrorism agents, including anthrax. To obtain human antibodies that neutralize anthrax toxin, libraries were panned against recombinant anthrax toxin proteins PA83 or against purified PA63, prepared by trypsin cleavage from PA83. Phage from various panning rounds were screened against PA83 and PA63 by ELISA and a panel of human antibody Fab fragments that bind anthrax proteins was generated. Relative affinities were assessed using a binding inhibition assay. Fabs were assayed for ability to neutralize anthrax toxin activity *in vitro*. Fourteen of sixteen anti-PA83 Fabs tested showed potent neutralizing activity, several at equimolar concentrations relative to PA83. One of three Fabs against PA63 neutralizes in sub-stoichiometric amounts, and may be acting at the level of heptameric pore structure. In rodent survival studies, these antibodies have demonstrated potent neutralization against recombinant toxin challenge. These antibodies may be useful therapeutically before or after exposure to anthrax toxin. Antibodies against additional category A bioterrorism agents isolated from these libraries will also be described.

[10.15–10.45]

Attenuation of Pseudomonas aeruginosa-induced mortality in immunodeficient and immunocompetent mice by anti-flagellin antibodies

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Pseudomonas aeruginosa (Pa) is a nosocomial pathogen that is associated with significant morbidity and mortality in burn patients, patients in intensive care units, cystic fibrosis patients and very low birth weight infants. The increase in resistance of Pa to currently available antibiotics lays credence for the development of novel classes of anti-Pa therapeutics to combat Pa infections. Such a class of potential anti-Pa agents are antibodies directed at critical Pa virulence factors.

Flagellin protein which constitutes flagellum, represents a key Pa virulence factor since it is centrally involved in bacterial motility, invasion into inflammatory cells (eg. epithelial cells) as well itself being pro-inflammatory. To investigate the role of Pa flagellin as a putative target for antibody neutralization, rabbit polyclonal IgG fractions raised against the N'-terminal domains of Pa flagellin types a and b were tested in *in-xvitro* assays and in *in-vivo* models of Pa infections.

In *in-vitro* invasion studies using A549 cells, a human lung epithelial cell line, pre-neutralization of Pa01 with anti-Pa flagellin type b IgG (1–250 μg) caused a dose-dependent inhibition of bacterial invasion with $\sim$ 75% inhibition at 250 μg IgG. A similar inhibitory effect was observed with antibodies raised against flagellin type a using a PAK bacterial strain. To validate Pa flagellin as a bona-fide target for putative antibody therapy, two lethal models of Pa infection were established. Firstly, CB57/Bl mice were rendered neutropenic by cyclophosphamide and challenged with intra-peritoneal (i.p.) Pa01 at doses of 104–106 cfu's per mouse. At all infectious doses, treatment of mice with 3 doses of 0.5 mg anti-Pa flagellin IgG at -1 hr, $+4$ hr and $+18$ hr totally suppressed mortality as compared to PBS or to non-relevant antibody treated groups. Significant protection was observed also at lower IgG doses. Secondly, using a combination of a localized burn injury followed by sub-cutaneous Pa administration in CB57/Bl mice, daily administration of 0.3–0.5 mg anti-Pa flagellin IgG prevented mortality associated with Pa infection of the burn.

These data would indicate that antibodies raised against Pa flagellin represent a novel approach to combat infection and mortality associated with Pa. Experiments are currently in progress aimed at generating fully human monoclonal antibodies to Pa flagellin types a and b using XTL's proprietary Trimera technology. These antibodies will be validated using the described animal models and *in-vitro* tools.