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[11.00–11.30]
Human monoclonal antibodies against viral diseases: A clinical perspective
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Abstracts not provided.

[11.30–12.00]
An influenza N1 neuraminidase-specific monoclonal antibody protects animal against live challenge with homologous H5N1 virus
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H5N1 avian influenza continues to be a potential pandemic threat. Several vaccine candidates based on potentially pandemic influenza strains and antiviral drugs have been tested in preclinical studies. The data obtained so far have shown some promise, but have also revealed some shortcomings of both approaches. We have identified and characterized an H5N1 neuraminidase-specific monoclonal antibody which specifically inhibits N1 neuraminidase activity of highly pathogenic avian influenza (HPAI) strains from clades 1 and 2. We have shown the protective efficacy of this antibody in animal challenge models using homologous virus. Specific and effective inhibition of N1 NA could make this MAb a useful therapeutic tool in the treatment of human infection, in particular with oseltamivir- and zanamivir-resistant strains of HPAI. This MAb could also become a useful diagnostic tool for typing H5N1 suspected human isolates in conjunction with other diagnostic approaches.

[12.00–12.20]
Isolation of broadly cross-neutralising and protective monoclonal antibodies against influenza virus
Mark Throsby
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Avian influenza viruses such as H5N1 are currently panzootic and pose a pandemic threat. These viruses are antigenically diverse and protective strategies need to cross protect against diverse viral clades. We isolated a panel of cross-reactive neutralising antibodies against H5N1 and multiple other subtypes of influenza A. The epitope of these antibodies was identified in the fusion stem domain of HA. The most potent antibody (CR6261) was protective in mice when given before and after lethal H5N1 or H1N1 challenge and could be used as a broad spectrum agent for prophylaxis or treatment of human or avian influenza infections without prior strain characterization.

[12.20–12.40]
Wide-spectrum, protective anti-β-glucan antibodies: Inhibitors of microbial virulence
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Glycoconjugate vaccines made up by an algal or bacterial β-glucan (laminarin or curdlan) and a carrier protein, protect against infections by different human opportunistic pathogenic fungi and induce antibodies capable of inhibiting fungal growth [1,2] and other unpublished data. We have called these vaccines "cross-kingdom or universal" since vaccine antigen and vaccine target belong to different kingdoms [3,4]. Directly inhibitory antimicrobial antibodies, which can in principle exert their protective activity without the help of immune system, are of obvious interest in this era of antimicrobial drug resistance and immunosuppres-

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sion. Moreover, they raise interesting basic question concerning mechanism of antimicrobial activity.

To try to unravel this mechanism we generated a number of murine anti- β-glucan mAbs some of which were modified as human chimeric antibodies in plants. Particularly, two mAbs (2G8 and 1E12) with identical CDR sequences but belonging to IgG2b and IgM, respectively, and both efficiently binding the fungus *Candida albicans* (taken as test model), markedly differed in their capacity to passively vaccinate against the fungus. Namely, the IgG2b was highly protective whereas the IgM was scarcely or non-protective at all neither in a systemic nor in a mucosal infection model. Affinity binding studies , ELISA inhibition assays and analysis by glycome arrays showed that the mAb 2G8 recognized with the highest affinity oligosaccharides with β1–3 configuration (tetraose to heptaose as minimum length) whereas the mAb 1E12 recognized efficiently both β1–3 and β1–6 oligosaccharide configurations. Importantly, the IgG2b mAb efficiently inhibited the in vitro growth of the hyphal , virulent forms of *C.albicans*, and blocked hyphal adherence to a mammalian epithelial cell line, two properties not shared by the IgM mAb. Since surface-exposed glucan-associated cell wall proteins (GAP) are likely targets for growth inhibition of fungal cells, we examined cell wall extracts and secretory material for the presence of mAb2G8-reactive, mab1E12-unreactive GAP. Two of them were identified as the GPI-anchor proteins Als3 and Hyr1, both previously characterized as critically involved in fungal adherence, biofilm formation, invasion of host tissues and hyphal growth. Overall, our data point to the critical function of antibody isotype in the mechanism of the direct, non immunoeffector-mediated, fungistatic activity of anti-β-glucan antibodies. They also support the notion that GAP critical for virulence expression are the biological target of anti-β-glucan protective antibodies.

References

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[12.40–13.00]

Construction and biological characterization of an anti-Venezuelan equine encephalitis virus humanized antibody

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Venezuelan equine encephalitis virus (VEEV) is an important mosquito-borne pathogen in humans and a potential biological warfare agent of concern. The murine monoclonal antibody 1A4A1 can strongly neutralize VEEV and is a good candidate for development of humanized antibody. Humanization of 1A4A1 variable domains was achieved by a complementarity-determining region grafting approach. The humanized 1A4A1 variable domains were further grafted onto human heavy and light chain constant domains to assemble the whole antibody gene. Meanwhile, a foot-and-mouth-disease virus-derived 2A self-processing sequence was introduced between heavy and light chain DNA sequences to express a full-length antibody from a single open reading frame driven by a single promoter in an adenoviral vector. The humanized 1A4A1 antibody was expressed in mammalian cells and purified by protein L affinity column. SDS-PAGE analysis showed that the heavy and light chains of the humanized antibody were cleaved completely. ELISA and plaque reduction assay showed that the humanized antibody retained high VEEV-binding specificity and VEEV-neutralizing activity comparable to its parent 1A4A1. Passive immunization of the humanized 1A4A1 antibody in mice (50 μg/mouse) 24 h before virulent VEEV challenge (100 $\times$ LD50) by subcutaneous route provided 100% protection against VEEV-induced mortality. This humanized 1A4A1 antibody may have prophylactic or therapeutic potential against VEEV infection in humans.