Session 3: Infectious diseases

Wednesday 25 April 2001, Moderator: Professor K. James

[09.00–09.30]

Recognition by human monoclonal antibodies of free and complexed peptides representing the prefusogenic and fusogenic forms of HIV-1 gp41

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Human immunodeficiency virus type 1 (HIV-1) entry into target cells appears to be triggered when two heptad repeat regions in the ectodomain of gp41 associate, converting the prefusogenic form of gp41 to a fusogenic form. Peptides from these two heptad repeat regions, designated N51 and C43, form a coiled coil consisting of an fÁ-helical trimer of heterodimers which approximates the core of the fusogenic form of gp41. To understand the antigenic structure of gp41 in these two configurations, and to examine the specificity of anti-gp41 antibodies produced by HIV-1-infected individuals, human anti-gp41 monoclonal antibodies (mAbs) were tested for their reactivity against N51, C43, and the complex formed by these peptides. The human mAbs were generated via fusion of a heteromyeloma with EBV-transformed peripheral lymphocytes derived from HIV-1-infected individuals. Of 11 mAbs, 7 reacted with the complex but with neither of the parent peptides. These mAbs reacted optimally with the N51-C43 complex prepared at a $1:1$ ratio and appeared to recognize the fusogenic form of gp41 in which the two heptad repeat regions are associated to form coiled coil.The existence of antibodies from HIV-1-infected humans that exclusively recognize the N51-C43 complex constitutes the first proof that the coiled-coil conformation of gp41 exists in vivo and is immunogenic. Two of the 11 mAbs were specific for the hydrophilic loop region of gp41 and failed to react with either peptide alone or with the peptide complex, while the remaining 2 mAbs reacted with peptide C43. One of these two latter mAbs, 98-6, also reacted well with the equimolar N51-C43 complex, while reactivity with C43 by the other mAb, 2F5, was inhibited by even small amounts of N51, suggesting that the interaction of these peptides occludes or disrupts the epitope recognized by mAb 2F5. Mabs 98-6 and 2F5 are also unusual among the mAbs tested in their ability to neutralize multiple primary HIV isolates, although 2F5 displays more broad and potent activity. The data suggest that anti-gp41 neutralizing activity is associated with specificity for a region in C43 which participates in complex formation with N51. Serum Abs specific for C43 are present in all HIV-infected individuals, but Abs that inhibit the ability of N51 to complex with C43, a step which may block the infectious process, are present in less than 5% of patients' sera. Characterization of the Abs with inhibitory activity may lead to design of immunogens corresponding to the C43 region that would be valuable for vaccine development.

[09.30–09.50]

Prevalence of antibody against tetanus and diphtheria in Egyptian population

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The aim of this study was to evaluate tetanus and diphtheria immunity (serioepidemiology) for the first time in Egypt. The level of antitetanus and antidiphtheria must be cheeked annually and compared to developed countries to decide if given populations need revaccination. Tetanus and diphtheria antitoxin levels were measured by means of the immunoenzymatic test (ELISA) in serum samples of 7000 females and males subjects of all ages from Cairo, Giza, Gharbia, Sohag, Kena. The subjects were divided into groups according to sex and age. More than 90% of newborn after the introduction of routine vaccination against tetanus and diphtheria had antitetanus and antidiphtheria levels above the minimum protective level (> 0.01 IU/ml). Our study indicated the effectiveness of tetanus and

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diphtheria vaccination programs in a majority of individuals. After adjusting for confounding effects in logistic regression tetanus and diphtheria immunity in those > 40 years was significantly higher in participants from Cairo and Giza than other locations. Generally, significant differences between males and females were observed. The complete statistical analysis and discussion will be presented.

[09.50–10.10]

Evaluation of human monoclonal antibodies to HCV in the HCV-Trimera model: a mouse model for HCV infection

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There are immediate needs for effective therapies for preventing and managing HCV infection. Therapeutic human antibodies to HCV that are able to reduce HCV viral load could serve either as a stand-alone or as an adjunct treatment in patients with chronic infection. Additionally, antibodies may be useful to prevent re-infection following liver transplantation or prevent infection following accidental exposure to HCV. Development of neutralizing antibodies to HCV was hampered by the lack of in vitro systems as well as suitable animal models to screen for effective antibodies. We have developed the HCV-Trimera mouse model, in which human liver fragments infected with HCV are transplanted into immunosuppressed mice. Viremia is followed by quantitation of HCV-RNA in sera of these mice. The presence of (-) strand HCV-RNA confirms viral replication in engrafted liver. This model has been used to evaluate the ability of anti HCV human monoclonal antibodies to reduce viral load and to inhibit HCV infection. Using peripheral B cells isolated from individuals infected with HCV genotype 1b, human monoclonal antibodies (HMAbs) to the HCV envelope protein (E2), were generated and characterized. These antibodies were tested in vitro for their affinity to E2 and for their ability to immunoprecipitate viral particles from sera of HCV infected patients. They were further tested in vivo in the HCV-Trimera mouse model. The antibodies were effective in inhibiting HCV infection of human liver fragments as measured by reductions in mean viral load and the percentage of HCV positive animals as compared to control groups. Furthermore, administration of HMAb HCV-AB 68 to HCV-Trimera mice with established viremia resulted in significant reduction of the mean viral load as well as reduction of the percentage of positive animals suggesting a possible role in treatment. Thus, the HCV-Trimera mouse model offers a powerful tool for simulating human HCV infection and for evaluating therapeutic antibodies. Our results indicate the feasibility of using HMAbs to HCV for prevention and treatment of HCV infection.

[10.10–10.30]

Hyperphage, antibodies from preimmunised donors, and new expression formats

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We show that the number of antibody fragments (scFv) presented on filamentous phage particles generated with antibody display phagemids can be increased by more than two orders of magnitude by using a novel helperphage (hyperphage) (*Nature Biotechnol.* **19**, 2001, 75–78). Hyperphage are of wildtype pIII phenotype, thus are able to infect F+ E. coli cells with high efficiency, but they do not carry a functional pIII gene, thus rendering the phagemid encoded pIII::antibody fusion as the sole source of pIII in phage assembly. This resulted in a dramatic increase of the fraction of phage particles carrying an antibody fragment on their surface. Thus, hyperphage is particularly useful in stoichiometrical situations where the chances of a single phage to meet the antigen are low. These situations are typically given in the first panning step, either when very large libraries are used, and/or when antigen is present in low concentrations, e.g. when panning on cell surfaces or 2D gel spots. Further, the use of hyperphage will decrease the chances to loose variability among the binders in the first panning step, and

will open new opportunities for proteomics. A novel form of bivalent and bispecific disulfide-stabilized Fv antibody fragments has been produced by secretion of three polypeptide chains into the periplasm of *E. coli*. These dsFv-dsFv' molecules consist of two different disulfide-stabilized Fv antibody fragments connected by a flexible linker peptide (*Protein Engineering* **13**, 2000, 725–734). This design may be of particular value for therapeutic in vivo applications since increased flexibility and improved stability is expected to be combined with minimal immunogenecity. Antibodies with therapeutic potential can be isolated from immunised donors using phage display. We used this approach to isolate antibody fragments binding to the surface of intact Hantavirus particles. Hantavirus is the causative agent of Hemorrhagic Fever with Renal Syndrome (HFRS), a life threatening disease with a worldwide distribution. Most cases are reported from China, however, increasing numbers of cases are reported from southern Europe as well. We used blood samples from patients which had survived the infection and constructed antibody display libraries from lymphocyte RNA. A panel of different antibodies to hantavirus (HTN) surface glycoproteins were obtained. For the expression of complete IgG molecules, we used a cassette baculovirus vector system which allows the direct insertion of the antigen binding regions obtained by phage display. Most of the isolated antibodies to hantavirus glycoproteins G1 and G2 showed neutralising effects in the plaque reduction neutralisation test. Renal cell carcinoma (RCC) generally has a poor prognosis since, once the diagnosis is made, metastases are already present in 30–40% of all patients while 50% develop them at a later date. At the present time, no efficient systemic treatment for metastatic RCC is available. Searching for new strategies in the targeted therapy of metastatic renal cell carcinoma (RCC), we generated several different recombinant antibodies derived from the monoclonal antibody 138H11 directed against human gamma-glutamyltransferase (GGT). 138H11 targets RCC in vivo due to a different cellular antigen localization compared to normal tissue (*Cancer Res.* **60**, 2000, 6089–6094). Monomeric single-chain Fv antibody fragments, diabodies and triabodies were obtained by constructing linker variants consisting of 18, 10, 8, 5, 3, 2, 1 and zero amino acid residues connecting the C-terminus of the heavy with the N-terminus of the light chain variable domain, thereby demonstrating the influence of the linker length on the molecular architecture and the antigen binding activities. Furthermore, two disulfide bond-stabilized Fv fragments (dsFvs) were generated utilizing two different pairs of complementary framework positions of VH and VL distant from the CDRs for disulfide stabilization. The various molecular formats will be used to identify the fragment with optimal pharmacokinetic parameters.