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P.4.04.37 Enteroviral infections and idiopathic dilated cardiomyopathy: Is there a causal relationship?

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Idiopathic dilated cardiomyopathy (IDC) is an end-stage heart disease of unknown cause. Heart transplantation is the only possibility for final therapy. IDC is likely to be a late outcome of myocarditis, a disease in which enteroviruses are known to be the main causative agents.

In order to study the role of enteroviruses in the pathogenesis of IDC, we collected sera, faecal swabs, throat swabs and multiple biopsy samples of explanted hearts from 77 patients during heart transplantation.

Thirty-five patients were diagnosed with IDC and 42 patients suffered from different kinds of heart diseases. No enteroviruses could be isolated from the throat swabs, the faecal swabs or the biopsy samples of the heart transplantation patients. A nested general primer-mediated enterovirus-specific polymerase chain reaction (PCR) was used to screen for the presence of enteroviral genomes in 856 heart biopsy specimens from 77 patients. The PCR primers in this assay are complementary to highly conserved sequences within the enteroviral 5'-non-translated region. An indirect immunofluorescence assay (IFA) was used to screen the sera for the presence of heart-specific autoantibodies.

Enterovirus RNA was not detected in any of the biopsy specimens. No heart-specific autoantibodies could be detected in any of the sera.

On the basis of these findings, we conclude that there is no enteroviral persistence in patients with IDC. Enteroviruses could be involved in the pathogenesis of IDC by triggering a cardiac-specific autoimmune response. However, we could not detect any heart-specific autoantibodies in sera from patients with end-stage heart disease. More experiments are required to find out whether autoimmunity actually plays a role in IDC.

P.4.04.38 Identification of an immunodominant neutralising and protective epitope from measles virus fusion protein using acute post-infection human sera

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Introduction: An immunodominant domain of the measles virus F protein was identified by mapping with acute post-infection sera and a functional role for this region was further analysed using a rodent model of MV infection.

Methods: Polyclonal sera obtained from African children with acute measles was used to screen a panel of 15-mer overlapping peptides representing the sequence of measles virus fusion (F) protein. To further investigate a functional role the for identified antigenic peptides, *in vivo* immunogenicity and passive protection studies were carried out in two strains of mice.

Results: An immunodominant antigenic region from the F protein (p32 aa 388–402) was recognised by 100% of serum samples tested: its sequence was found to represent an amino acid sequence within the highly conserved cysteine-rich domain of the F protein of paramyxoviruses. Epitope mapping of this peptide indicated that the complete 15 amino acid sequence was necessary for high affinity interaction with anti-MV antibodies. Immunisation of two strains of mice with the p32 peptide indicated that it was immunogenic and could induce anti-peptide antibodies which cross-reacted with and neutralised MV infectivity *in vitro*. Moreover, passive transfer of anti-peptide antibodies conferred significant protection against fatal rodent-adapted MV encephalitis in susceptible mice.

Conclusion: These results indicate that this peptide represents a candidate for inclusion in a synthetic peptide-based measles vaccine.

P.4.04.39 Virus-induced type-1 cytokine response in IL-12 deficient mice

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Introduction: Both IL-12 and IFN-γ have been implicated as principal inducers of type-1 immune responses required for the elimination of intracellular pathogens, such as viruses. We examined the *in vivo* role of both cytokines in the development of cytokine responses and antiviral resistance during coronavirus-induced hepatitis in a mouse hepatitis virus (MHV) model.

Results: Absence of IFN-γ-function in mice with a targeted disruption of the IFN-γ receptor α chain gene (IFN-γR–/–) resulted in increased susceptibility to coronaviral hepatitis that was associated with augmented viral replication and increased hepatocellular injury. IL-12 treatment failed to restore protective immunity in IFN-γR–/– mice but significantly protected MHV susceptible C57BL/6 mice against lethal infection; although less than IFN-γ-treatment. These findings illustrate the pivotal antiviral role of IFN-γ-in protection against acute coronavirus-induced hepatitis. The mutant mice showed a type-1 lymphokine response characterized by the normal high IFN-γ and the low IL-4 production. Unlike MHV-infected wild-type mice, however, the mutant IFN-γR–/– mice showed no increase of IL-12 p40 gene expression, similar as in naive animals. We therefore examined antiviral responses in gene-targeted IL-12 knockout mice, which normally produce a polarized type 2 cytokine response characterized by strongly impaired IFN-γ-production and significantly higher levels of IL-4 in other systems. Surprisingly, MHV-infected IL-12p35 and IL-12p35/p40 deficient mice showed no increased susceptibility. Strikingly, they also generated a type 1 cytokine response.

Conclusions: Our data demonstrate that in the absence of IL-12 gene expression or defective IFN-γR function virus-induced IFN-γ-production without increased T cell IL-4 synthesis can occur. It is interesting to consider which mechanism mediates the virus-induced type-1 responsiveness. Theoretically both IL-12 and IFN-γ are redundant. We are currently studying the effect of antibody-mediated IFN-γ depletion in MHV-infected IL-12 deficient mice.

P.4.04.40 Influence of MHC class I and class II (mis)matching between donor and recipient on the cytomegalovirus induced accelerated graft arteriosclerosis in a rat aorta transplant model

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Introduction: Accelerated transplant arteriosclerosis or chronic rejection, characterized by persistent intimal thickening, is the major life threatening complication in long-term surviving organ transplant recipients nowadays. Cytomegalovirus (CMV) infection is one of the risk factors recognized in clinical studies to be involved in the development of these lesions. Also in animal studies CMV infection accelerates the development of allograft arteriosclerosis (Li et al., Transpl. Proc., 1995; 27: 3552). Clinical data on the importance of various MHC-loci in this CMV induced accelerated development of vascular allograft lesions are inconclusive sofar. Therefore we investigated the combined effect of class I and class II MHC (mis)matching between donor and recipient and CMV infection on the development of graft arteriosclerosis using defined rat combinations in an aortic transplant model.

Materials & Methods: Male Lewis (RT-1^l; class I^l, class II^l) and WAG (RT-1^u; class I^u, class II^u) rats were used as donors. Male RP (RT-1^p; class I^u, class II^u) were used as recipients. Aortic transplantation (Tx) from Lewis to RP resulted thus in class I disparity, and the combination of WAG and RP in a class II difference. One day after Tx all rats received 5 Gy total body irradiation. To study the role of CMV infection half of the recipient rats received rat CMV (RCMV) infection at the same day. Animals were sacrificed at 7, 28 and 90 days after Tx, and aortic grafts were taken out for further histological analysis, including immunostaining for various lymphocyte and macrophage subset and activation markers, as well as quantification of neointima formation.

Results: Chronic rejection, characterized by intima thickening and inflammation, developed in both strain combinations, also without RCMV infection. However, vascular lesions were much more severe at 90 days in the class II disparate combination (WAG to RP), than in the class I disparate combination (Lewis to RP). RCMV infection, on the other hand, did accelerate the development of vascular pathology in the class I, but not in the class II differing combination. Subset and activation marker analysis revealed the characteristic pattern of inflammation, however no major differences were observed between the various experimental groups.

Conclusions: Analysis of the combined effect of MHC locus (mis)matching and CMV infection in a rat aorta transplant model revealed that both class I and class II mismatching evoked chronic rejection pathology. Additional CMV infection, however, resulted only with class I disparity between donor and recipient in accelerated graft arteriosclerosis. These findings could not be explained by differences in inflammation patterns, since these were similar in all groups.

P.4.04.41 Attenuated recombinant *Bordetella pertussis* as live vaccines for the protection against homologous and heterologous diseases

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Introduction: Infection often provides stronger protection and of longer duration against subsequent infections than vaccination with defined antigens. Therefore, several live attenuated pathogens have been developed as vaccine candidates. Expression of heterologous antigens in such strains may yield vaccines able to protect against several diseases simultaneously. Although several enteric

pathogens have been constructed for oral delivery, little effort has been devoted to the design of live attenuated vectors to specifically target the respiratory mucosa, which presents several advantages over the oral route. Here, we explore the potential of attenuated recombinant Bordetella *pertussis* to protect against pertussis and other bacterial and parasitic infections after a single intranasal administration.

Materials and Methods: The *B. pertussis* filamentous haemagglutinin (FHA) was used to expose heterologous antigens at the surface of the microorganism. The *Schistosoma mansoni* glutathione S-transferase (Sm28GST) and the *Neisseria meningitidis* transferrin-binding protein 2 (Tbp2) were genetically fused to FHA. The hybrid genes were recombined into the *fha* locus of the *B. pertussis* wild type strain BPSM and the attenuated strain BPRA, lacking the pertussis toxin gene, the major virulence determinant. OF1 and Balb/C mice were intranasally infected with the different strains, analysed for *B. pertussis* colonisation and for immunogenicity of the bacterial strains and hybrid antigens. Finally, the mice were tested for protection against *B. pertussis* and *S. mansoni* challenge.

Results: Immunoblot analyses indicated that the FHA-Sm28GST and FHA-Tbp2 were surface exposed and secreted. A single infection of the recombinant bacteria yielded high levels of mucosal antibodies in the bronchoalveolar lavage fluids against the heterologous antigens. Interestingly, the deletion of the pertussis toxin gene resulted in a substantial increase in serum anti-FHA antibodies after nasal administration. A similar increase in serum antibodies was also obtained against the heterologous antigens after infection with the recombinant attenuated strains. Subsequent challenge with either *B. pertussis* or with *S. mansoni* indicated that the attenuated recombinant strain protected against pertussis and the parasite infection with respect to worm burden and egg charge.

Conclusion: A single intranasal infection with attenuated recombinant *B. pertussis* protects mice against pertussis and a heterologous infection. The deletion of the pertussis toxin gene increases the immunogenicity of FHA and the fused heterologous antigens, indicating for the first time that attenuation can enhance immunogenicity. Finally and in contrast to previous believes, pertussis toxin is not required for protection by attenuated live *B. pertussis*.

P.4.04.42 The practical value of the antiinfluenza antibodies electrokinetic potential determination

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For effective prophylaxis of influenza it is important to estimate the antigenic structure of currently circulating viruses and to determine the most epidemically dangerous strain. It is known some serological and immuno-epidemiological approaches to answer these questions. But sometimes it is very hard to isolate the circulating virus from population and to make exact epidemiological prognosis.

In this study, sera from a blood donors were tested for the strain-specific antiinfluenza antibodies (AB) with positive (AB+) and negative (AB-) electrokinetic potential. The electrokinetic potential of AB was estimated using free-flow electrophoresis method.

We ascertained that the correlation between titers of the strain-specific AB: (AB+/AB−) in human sera changed in dependence of the season of the year. In the same serum specific AB to different influenza strains had different value of the index (AB+/AB−). In serum AB from vaccines with polyvalent influenza vaccine these indexes was smaller than in serum AB from not vaccinated donors. The interrelation between average value of the index (AB+/AB−) for concrete strain-specific AB in human sera and the degree of epidemic danger of corresponding viral strain was noticed. Average titer of the strain-specific AB+ in human sera correlated with the level of circulation of the viruses with corresponding antigenic structure in the examined human population.

The obtained results permit to use the study of electrokinetic characteristics of antiviral antibodies as additional express-method for the more exact evaluation of influenza epidemic situation.

P.4.04.43 Some immunogenicity determinative factors (IDF) in influenza viruses

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Information about IDF in pathogens is necessary for successful vaccines constructing and vaccine strains selection. Investigation of this question was the main purpose of our work.

The groups of standard, experimentally received and recently isolated influenza virus (I.V.) strains were examined. Viruses inside each group possessed identical antigenic structure but differed by immunogenicity. The methods of RNA sequencing, SDS-PAAG- and free-flow electrophoresis were used.

One of the IDF in I.V. is structure of the viral membrane glycoprotein – hemagglutinin (HA): we ascertained that amino acid substitutions in the site of receptor-binding pocket of HA-molecule and that site which provided initial adsorption of the virus on the cell (140–145 positions of HA-1) changed I.V. immunogenicity. Besides viral immunogenicity correlated with the level of proteolytic cleavage of viral nucleoprotein (NP) and relative contents of viral polypeptides M and NP in the virions. I.V. population is heterogenous by the sign of virion's electrokinetic potential (EKP). Immunogenicity of I.V. strain correlated with the relative contents of virions with certain EKP in the viral population. According to obtained data the markers of the augmented immunogenicity in I.V. are: 1) high relative contents of polipeptide M in the virions; 2) low level of proteolytic cleavage of NP; 3) low contents of virions with positive EKP and high contents of virions with negative EKP in population.

Thus, the genetic determination of increased or reduced immunogenicity sign in influenza viruses was shown. Received results allowed to work out the I.V. immunogenicity evaluation criteria for express-screening of I.V. vaccine strain candidates.

P.4.04.44 EBV-specific cytotoxic T-cells (CTL) recognize the immediate early transactivators Zta (BZLF1) and Rta (BRLF1) of EBV

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Introduction: Latent LBV-infection is effectively controlled by cellular immune mechanisms. We were able to show that the transactivator proteins Zta (BZLP1) and Rta (BRLF1) expressed at the beginning of the lytic cycle can act as targets for EBV-specific CTLs.

Mat. and Methods: Panels of overlapping peptides covering the entire amino acid sequence of Zta and Rta were synthesized and used to induce and analyse specific CTL responses in several HLA-typed EBV-positive donors. Specific lysis of target cells was tested in ^{51}chromium-release assays.

Results: Using the Zta peptides in experiments with several donors we were able to identify two epitopes recognized by CTLs. The first epitope could be mapped to an octameric sequence between amino acid positions 190 and 197 of the Zta protein and proved to be restricted by HL-B8 (Bogedain et al., J Virol 69: 4872–79). A second epitope overlaps with the first one but has a length of 12 amino acids (187–198) and is restricted by HLA-Cw6. No similarity was found with other HLA-Cw6 restricted epitopes published so far. Using 75 overlapping peptides spanning the entire Rta sequence we found 9 different CTL epitopes, that are distributed over the entire protein. Epitope 1 is located between positions 28 and 37 and restricted by HLA-A24. Another HLA-B18 restricted epitope could be mapped to the same region (25–38). The other epitopes were proved to be restricted by HLA-A2 (225–239), HLA-A3 (145–159), HLA-A11 (129–143), HLA-B61 (529–543) and HLA-Cw4 (393–407). Of two of the epitopes the HLA-restriction has not yet been identified. Experiments using recombinant vacciniaviruses expressing Zta and Rta showed for the majority of CTL clones that they recognize endogenously processed peptides just as well as exogenously loaded synthesized ones. On the other hand we also found clones that do recognize peptide labelled target cells but don't seem to recognize vaccinia infected target cells.

Conclusion: Additionally to the immune mechanisms active during latent infection a CTL response against immediate early-proteins of the lytic cycle could provide an efficient way of eliminating EBV-infected cells before they start to produce progeny virions. This phenomenon may be an important part of the EBV-immune surveillance which is responsible for the low incidence of EBV-associated diseases despite the very high prevalence and the B-cell immortalizing properties of this virus.

P.4.04.45 Genetic influences on the induction of eosinophilic pathology during lung viral infection

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Introduction: BALB/c mice sensitised to the major surface glycoprotein (G) of respiratory syncytial virus (RSV) develop lung eosinophilia after intranasal challenge with whole virus. This pattern of pathology is accompanied by the development of T cells displaying intracellular Th2 cytokines.

Methods: We have assessed the sensitivity to eosinophilic pathology in 19 different inbred strains of mice. Mice were scarified with G and then challenged intranasally 2 weeks later with whole virus. 7 days after challenge cells were recovered from the lungs by lavage and cell subsets and cytokine expression determined by flow cytometry.

Results: Mice with the H-2^d haplotype consistently developed eosinophilia when sensitised with G and challenged with RSV whereas mice with the H-2^k haplotype did not. This lack of eosinophilic pathology is not due to the absence of an immune response to RSV as a similar CD4+ and CD8+ cellular efflux was observed in the bronchoalveolar space. H-2^k mice also developed similar virus specific antibody responses as assessed by ELISA. Mice with a C57BL background (BL/10 and BL/6) also responded to virus infection but failed to develop lung eosinophilia. By intracellular staining we were able to show that