First annual meeting
Belgian Society for Virology
Friday, November 8, 2013
The Royal Academies for Science and the Arts
Hertogsstraat/Rue Ducale 1, Brussels

8h15	**"Welcome & launch of Belgian Society of Virology"** ***Hans Nauwynck***
8h30 – 12h30	**Pathogenesis/immune response/immune evasion - viral diseases** ***Chairmen: Alain Vanderplasschen & Herman Favoreel***
8h30 – 9h15	Pathogenesis - keynote lecture "Nuclear entry of retroviruses" *Zeger Debyser*
9h15 – 9h30	Pathogenesis - selected talk 1 "An essential role for gamma-herpesvirus latency-associated nuclear antigen homolog in an acute lymphoproliferative disease of cattle" *L. Palmeira, O. Sorel, W. Van Campe, C. Boudry, S. Roels, F. Myster, A. Reschner, P. Coulie, P. Kerkhofs, A. Vanderplasschen & B. Dewals*
9h30 – 9h45	Pathogenesis - selected talk 2 "A novel mechanism of alphaherpesvirus immune evasion: the pseudorabies virus glycoprotein gD interferes with Natural Killer cell-mediated lysis of infected cells" *Korneel Grauwet, C. Cantoni, M. Parodi, A. De Maria, L. Moretta, M. Vitale & H. Favoreel*
9h45 – 10h00	Pathogenesis - selected talk 3 "Post-translational modification of the myxoma virus chemokine-binding protein M-T7 by a virally encoded alpha-2,3-sialyltransferase" *B. Boutard, S. Vankerckhove, K. Canis, N. Markine-Goriaynoff, M. Sarlet, R. Wattiez, P. Leprince, D. Desmecht, S. Haslam, G. McFadden, A. Dell, A. Vanderplasschen & L. Gillet*
10h00 – 10h15	Pathogenesis - selected talk 4 "The mechanism of behavioral fever induced by cyprinid herpesvirus 3 in common carp (*Cyprinus carpio L.*) *K. Rakus, P. Ouyang, J. Jazowiecka-Rakus, M. Ronsmans, M. Boutier, M. Forlenza, G. Wiegertjes, C. Becco, F. Farnir & A. Vanderplasschen*

10h15 – 10h30	Pathogenesis - selected talk 5 "Genomic analysis of Belgian G2P[4] rotaviruses reveals frequent reassortment with animal DS-1-like rotaviruses" *M. Zeller, V. Nuyts, E. Heylen, S. De Coster, M. Van Ranst & J. Matthijnssens*
10h30 – 11h00	**Coffee break**
11h00 – 11h15	Pathogenesis - selected talk 6 "DNA methylation: a positive regulator of viral telomerase RNA expression in Marek's disease virus?" *C. Mignion, S. Laurent, G. Dambrine, D. Rasschaert & B. Muylkens*
11h15 – 11h30	Pathogenesis - selected talk 7 "Evaluation of West Nile virus (WNV) NS3Thr249Pro mutation as a virulence marker in a SPF chicken model and in Carrion crows (*Corvus Corone*) *M. Dridi, C. Bahuon, D. Vangeluwe, T. van den Berg, S. Lecollinet & B. Lambrecht*
11h30 – 11h45	Pathogenesis - selected talk 8 "Genome-wide shRNA screening identifies new host factors involved in HIV-1 mediated CD4 down-modulation" *A. Landi, J. Vermeire, V. Iannucci, H. Vanderstraeten, E. Naessens, M. Bentahir & B. Verhasselt*
11h45 – 12h00	Pathogenesis - selected talk 9 "Establishment of feline intestinal epithelial cell cultures for the propagation and study of feline enteric coronaviruses" *L. Desmarets, S. Theuns, D. Olyslaegers, A. Dedeurwaerder, B. Vermeulen, I. Roukaerts & H. Nauwynck*
12h00 – 12h15	Pathogenesis - selected talk 10 "Study of the virulence of serotypes 4 and 9 of the orbivirus African horse sickness virus in two mouse models" *M. De la Grandière, F. Dal Pozzo, W. Zonta, A. Mauroy, A. Caij & E. Thiry*
12h15 – 12h30	Pathogenesis - selected talk 11 "High-throughput analysis of human cytomegalovirus genetic diversity reveals variation in coding capacity and extensive recombination" *S. Sijmons, K. Thys, M. Corthout, E. Van Damme, M. Van Loock, S. Bollen, F. Aerssens, M. Van Ranst & P. Maes*
12h30 – 13h30	**Sandwich lunch**
13h30 – 17h00	**Control of viral diseases (antivirals/vaccination)** ***Chairmen: Johan Neyts & Thierry van den Berg***
13h30 – 14h15	Control - keynote lecture "Feline enteric coronaviruses and feline infectious peritonitis virus, Jekyll and Hyde in cats" *Peter Rottier*

14h15 – 14h30	Control - selected talk 1 "A new method to deliver an attenuated vaccine against bovine leukemia virus in herds" *G. RamaRao, S. Rodriguez, G. Gutierrez, A. Debrogniez, N. Gillet, K. Trono & L. Willems*
14h30 – 14h45	Control - selected talk 2 "Towards a DNA-based live attenuated vaccine against the yellow fever virus and other flaviviruses" *K. Dallmeier, N. Mishra, D. Buh Kum, J. Zmurko & J. Neyts*
14h45 – 15h00	Control - selected talk 3 "Comparison HIV-1 envelope DNA vaccine candidates within three different animal models: guinea pigs, rabbits and cynomolgus macaques" *L. Heyndrickx, M. Borggren, L. Vinner, B. Andresen, B. Grevstad, J. Repits, T.Elvang, F. Martinon, N. Dereuddre-Bosquet, E. Bowles, G. Stewart-Jones, P. Biswas, G. Scarlatti, M. Jansson, G. Vanham, R. Le Grand, A. Fomsgaard*
15h00 – 15h30	**Coffee break**
15h15 – 15h30	Control - selected talk 4 "Rational design of an attenuated recombinant vaccine against Cyprinid Herpesvirus 3 using BAC cloning mutagenesis and in vivo imaging system" *M. Boutier, P. Ouyang, G. Fournier, Ronsmans M., Reschner A., Scohy S. & Vanderplasschen A.*
15h30 – 15h45	Control - selected talk 5 "SHe's a novel target for HRSV vaccination" *B. Schepens, L. Vande Ginste, S. De Baets, K. Sedyen, I. Rossey, P. Bogaert, B. Gilbert, P. Piedra, W. Fiers & X. Saelens*
15h45 – 16h00	Control - selected talk 6 "Treatment with anti-rabies VHH prevents or delays disease and mortality in mice depending on the time of treatment" *S. Terryn, A. Francart, S. Lamoral, A. Hultberg, P. Vanlandschoot, H. Rommelaere, A. Wittelsberger, M. Kalai & S. Van Gucht*
16h00 – 16h15	Control - selected talk 7 "Preventing the spread of foot-and-mouth disease virus with antiviral drugs" *D. Lefebvre, A. De Vleeschauwer, T. Willems, T. Rosseel, S. Van Borm, N. Goris, L. Murao, E. Kiss, D. Kollanur, A. Billiet, J. Swinne, J. Neyts & K. De Clercq*
16h15 – 16h30	Control - selected talk 8 "Bromodomain and extra-terminal (BET) proteins target Moloney Murine Leukemia Virus integration to transcription start sites" *J. De Rijck, C. de Kogel, J. Demeulemeester, S. Vets, N. Malani, F. Bushman, K. Busschots, S. Husson, R. Gijsbers & Z. Debyser*

16h30 – 16h45	Control - selected talk 9 "Synergistic activation of HIV-1 expression by compounds targeting the positive transcription elongation factor b (P-TEFb) and by inducers of the NF-□B signaling" *G. Darcis, A. Kula, S. Bouchat, K. Kabeya, N. Delacourt, C. Vanhulle, J. Gatot, D. Vaira, A. Burny, G. Herbein, N. Clumeck, O. Rohr, M. Moutschen, C. Rouzioux, S. De Wit & C. Van Lint*
16h45 – 17h00	Control - selected talk 10 "Antiviral agents to control classical swine fever epidemics: an epidemiologic and economic modeling study in a densely populated livestock area in The Netherlands" *R. Vrancken, J. Backer, J. Neyts & N. Gorix*
17h00 – 17h15	Control - selected talk 11 "Redistribution of HIV-based viral vectors for safer gene therapy" *S. Vets, C. Brendel, M. Grez, F. Bushman, Z. Debyser & R. Gijsbers*
17h00	**Reception**

All abstracts (the full text can be found on the BELVIR website):

Pathogenesis

1 The Tax protein and the minichromosome maintenance protein complex MCM2-7 affect cell replication and viral transcription
Barez P., Carpentier A., Boxus M. & Willems L.

2 The leader protein of Theiler's murine encephalomyelitis virus inhibits stress granule assembly by blocking PKR activation
Borghese F. & Michiels T.

3 Post-translational modification of the myxoma virus chemokine-binding protein M-T7 by a virally encoded alpha-2,3-sialyltransferase
Boutard B., Vankerckhove S., Canis K., Markine-Goriaynoff N., Sarlet M., Wattiez R., Leprince P., Desmecht D., Haslam S., McFadden G., Dell A., Vanderplasschen A. & Gillet L.

4 Downregulation of several cell surface markers in equine herpesvirus 1 (EHV1) infected equine mesenchymal stem cells is dependent on the expression of the viral protein pUL56
Claessen C., Favoreel H., Ma G., Osterrieder N., De Schauwer C. & Van de Walle G.

5 Characterization of messenger RNA termini in Schmallenberg virus and related Simbuviruses
Coupeau D., Claine F., Wiggers L., Beer M., Kirschvink N. & Muylkens B.

6 The ORF7-encoded accessory protein 7a of feline infectious peritonitis virus as a counteragent against interferon-alpha induced antiviral response
Dedeurwaerder A., Olyslaegers D., Desmarets L., Roukaerts I., Theuns S. & Nauwynck H.

7 Integrase interface mutant reveals importance of transportin-SR2-integrase interaction in HIV nuclear import
De Houwer S., Demeulemeester J., Thys W., Christ F. & Debyser Z.

8 Study of the virulence of serotypes 4 and 9 of the orbivirus African horse sickness virus in two mouse models
De la Grandière M., Dal Pozzo F., Zonta W., Mauroy A., Caij A. & Thiry E.

9 Establishment of feline intestinal epithelial cell cultures for the propagation and study of feline enteric coronaviruses
Desmarets L., Theuns S., Olyslaegers D., Dedeurwaerder A., Vermeulen B., Roukaerts I. & Nauwynck H.

10 Impact of latent murid herpesvirus 4 infection on the development of an anti-pneumovirus vaccine-induced respiratory immunopathology
Dourcy M., Machiels B., Desmecht D., Dewals B., Vanderplasschen A. & Gillet L.

11 Characterization of L* of different Theiler's virus strains
Drappier M. & Michiels T.

12 Evaluation of West-Nile virus (WNV) NS3Thr249Pro mutation as a virulence marker in a SPF Chicken model and in Carrion crows (Corvus corone)
Dridi M., Bahuon C., Vangeluwe D., van den Berg T., Lecollinet S. & Lambracht B.

13 Massive depletion of bovine leukemia virus proviral clones located in genomic transcriptionally active sites during primary infection
Gillet N., Gutiérrez G., Rodriguez S., de Brogniez A., Renotte N., Alvarez I., Trono K. & Willems L.

14 A novel mechanism of alphaherpesvirus immune evasion: the pseudorabies virus glycoprotein gD interferes with Natural Killer cell-mediated lysis of infected cells
Grauwet K., Cantoni C., Parodi M., De Maria A., Moretta L., Vitale M. & Favoreel H.

15 Human but not mouse hepatocytes respond to interferon-lambda in vivo
Hermant P., Demarez C., Mahlakoiv T., Staeheli P., Meuleman P. & Michiels T.

16 Rabbit colony infected with a bovine G6P[11] rotavirus strain
Heylen E., Schoondermark-van de Ven E., Zeller M., van den Hurk P., de Bruin W., Matthijnssens J. & Van Ranst M.

17 Role of the RhoA signaling pathway in cytoskeletal rearrangements induced by the alphaherpesvirus US3 kinase
Jacob T., Van den Broeke C., van Troys M., Ampe C. & Favoreel H.

18 Characterization of an infectivity assay for the human parvovirus B19 on the HepG2 hepatocarcinoma cell line
Kubler L., Caillet-Fauquet P., Baurin S., Timmerman D., Draps M. & Op de beeck A.

19 Genome-wide shRNA screening identifies new host factors involved in HIV-1 mediated CD4 downmodulation
Landi A., Vermeire J., Iannucci V., Vanderstraeten H., Naessens E., Bentahir M. & Verhasselt B.

20 Generation of trans-encapsidated Theiler's virus replicons
Lardinois C. & Michiels T.

21 Murid herpesvirus 4 ORF63 is not essential but contributes to virus replication and latency establishment
Latif M., Machiels B., Vanderplasschen A. & Gillet L.

22 LDV exacerbation of Fc receptor-mediated autoimmune blood diseases
Legrain S., Su D. & Coutelier J.

23 A gammaherpesvirus uses alternative splicing to regulate its tropism and its sensitivity to neutralization
Machiels B., Stevenson P., Vanderplasschen A. & Gillet L.

24 Experimental evidence of reassortant in Simbuviruses related to Schmallenberg virus, an emerging arbovirus in Europe
Marichal A., Baillieux P., Wiggers L., Kirschvink Nathalie, Muylkens B. & Coupeau D.

25 Bovine noroviruses in Belgium: from a molecular and evolutionary perspective
Mauroy A., Scipioni A., Daube G. & Thiry E.

26 DNA methylation: a positive regulator of viral telomerase RNA expression in Marek's disease virus?
Mignion C., Laurent S., Dambrine G., Rasschaert D. & Muylkens B.

27 Viral semaphorin encoded by Alcephaline herpesvirus 1 is a secreted glycoprotein that

is not essential that is not essential for malignant catarrhal fever induction
Myster F., Palmeira L., Sorel O., Vanderplasschen A. & Dewals B.

28 An essential role for gamma-herpesvirus latency-associated nuclear antigen homolog in an acute lymphoproliferative disease of cattle
Palmeira L., Sorel O., Van Campe W., Boudry C., Roels S., Myster F., Reschner A., Coulie P., Kerkhofs P., Vanderplasschen A. & Dewals B.

29 Study of the epigenetic control of chicken miR-126, a tumor suppressor microRNA downregulated in cells transformed and latently infected with Marek's disease virus
Parissi L., Gennart I., Mignion C., Decoster C., Coupeau D. & Muylkens B.

30 Is ther a link between the multiple activities of Theiler's virus Leader (L) protein?
Peeters M., Sorgeloos F. & Michiels T.

31 Mechanisms of entry of the human T-Lymphotropic virus type I and bovine leukemia virus into its host cells
Perike S., Van Hamme E., Nauwynck H. & Willems L.

32 The IL-10 homologue encoded by cyprinid herpesivirus 3 is essential neither for viral replication in vitro nor for virulence in vivo
Ouyang P., Rakus K., Boutier M., Reschner A., Leroy B., Ronsmans M., Fournier G., Scohy S., Costes B., Wattiez R. & Vanderplasschen A.

33 Interference of pseudorabies virus with Erk 1/2 signaling in T lymphocytes
Pontes M., Bienkowska-Szewczyk & Favoreel H.

34 Schmallenberg virus infection of pigs
Poskin A., Van Campe W., Mostin L., Vervaeke M., Caij B. & De Regge N.

35 The mechanism of behavioral fever induced by Cyprinid herpesvirus 3 in common carp
Rakus K., Ouyang P., Jazowiecka-Rakus J., Ronsmans M., Boutier M., Forlenza M., Wiegertjes G., Becco C., Farnir F. & Vanderplasschen A.

36 Immunological tools for the measurement of the immune response against respiratory viral infections of poultry (mainly AI & ND)
Rauw F., van den Berg T. & Lambrecht B.

37 Identification and characterization of new Sp1 sites located in the R region of the human T-lymphotropic virus 1 (HTLV-1) long terminal repeat
Robette G., Van Driessche B., Vanhulle C., Burny A., Rohr O. & Van Lint C.

38 Sensitivity and permissivity of cyprinus carpio to cyprinid herpesvirus 3 infection according to host ontogenesis
Ronsmans M., Boutier M., Szpirer C., Rougeot C., Vandecan M., Mélard C. & Vanderplasschen A.

39 Feline peripheral blood mononuclear cell cultivation: cell population evolution, FIV receptor expression & FIV replication
Roukaerts I., Olyslaegers D., Desmarets L., Theuns S., Dedeurwaerder A & Nauwynck H.

40 High-throuhgput analysis of human cytomegalovirus genetic diversity reveals variation in coding capacity and extensive recombination
Sijmons S., Thys K., Corthout M., Van Damme E., Van Loock M., Bollen S., Aerssens J., Van Ranst M. & Maes P.

41 Use of a new RT-qPCR assay for diagnosis of genetically heterogenous porcine group A rotaviruses in Belgium reveals a possible synergism with enteric bacteria
Theuns S., Desmarets L., Heylen E., Zeller M., Dedeurwaerder A., Van Ranst M., Matthijnssens J. and Nauwynck H.

42 Hepatitis E virus infection in wild boars and humans in Belgium
Thiry D., Mauroy A., Saegerman C., Brochier B., Thomas I., Linden A. & Thiry E.

43 An enterovirus 71 mouse model with central nervous system involvement
Tijsma A., Thibaut H., Franco D. & Neyts J.

44 HIV-1 Vpr N-terminus is critical for efficient Vpr de novo expression and infectivity in non-activated CD4+ T cells
Van Nuffel A., Impens F., Baeyens A., Vanhee S., Witkowski W., Vandermarliere E., Vanderstraeten H., Naessens E., Vanlandeghem K., Vermaut S., Moens K., Van Damme P., Gevaert K. & Verhasselt B.

45 Porcine, murine and human sialoadhesin (Sn/Siglec-1/CD169): portals for porcine reproductive and respiratory syndrome virus entry into target cells
Van Breedam W., Verbeeck M., Christiaens I., Van Gorp H. & Nauwynck H.

46 The ORF25 gene family of cyprinid herpesvirus 3 encodes non essential structural glycoproteins
Vancsok C., Jazowiecka-Rakus, Boutier M., Reschner A., Rakus K., Ouyang P. & Vanderplasschen A.

47 Comparison of ion torrent PGM and Illumina MiSeq next generation sequencing for influenza A virus quasispecies analysis
Van den Hoecke S., Verhasselt P. & Saelens X.

48 Dual role of the cellular cofactor CTIP2 in HIV-1 latency
Van Driessche B., Cherrier T., Vanhulle C., Delacourt N., Schwartz C., Van Lint C. & Rohr O.

49 Interferon-inducible Mx1 protein inhibits influenza virus by interfering with functional viral ribonucleoprotein complex assembly
Verhelst J., Parthoens E., Schepens B., Fiers W. & Saelens X.

50 Type I IFN is produced by HIV-1 infected primary CD4+ T cells
Vermeire J., Iannucci V., Naessens E., Van Landeghem K., Vanderstraeten H., Van Damme J. & Verhasselt B.

51 Pseudorabies virus isolates originating from Belgian domestic pigs and wild boar: genetic characterization and evaluation of their in vitro virulence
Verpoest S., Caij A. & De Regge N.

52 Glycoprotein 150 promotes sexual transmission of murid herpesvirus 4
Zeippen C., Vanderplasschen A., Stevenson P. & Gillet L.

53 Genomic analysis of Belgian G2P[4] rotaviruses reveals frequent reassortment with animal DS-1-like rotaviruses
Zeller M., Nuyts V., Heylen E., De Coster S., Van Ranst M. & Matthijnssens

Control

54 Defining and targeting the HTLV-1 Tax and PDZ proteins interactome
Blibek K., Fujii N., Legros S., Boxus M., Dewulf J., Zimmerman P., Kettmann R., Borg J., Dequiedt F. & Twizere J.

55 Small-molecule selective inhibitors of nuclear export (SINE) potently suppress HIV replication
Boons E., Vanstreels E., Vercruysse T., Shacham S., Landesman Y., Baloglu E., Tamir S., Pannecouque C. & Daelemans D.

56 Broad-spectrum anti-HIV activity of a llama single domain intrabody targeting Rev multimerization
Boons E., Vanstreels E., Vercruysse T., Li G., Pannecouque C., Vandamme A. & Daelemans D.

57 Rational design of an attenuated recombinant vaccine against Cyprinid herpesvirus 3 using BAC cloning mutagenesis and in vivo imaging system
Boutier M., Ouyang P., Fournier G., Ronsmans M., Reschner A., Scohy S. & Vanderplasschen A.

58 Checkpoints modulation by the human T-lymphotropic virus type 1 Tax protein
Carpentier A., Barez P., Boxus M. & Willems L.

59 Viral particles produced in presence of LEDGINs are impaired for infectivity
Christ F., Desimmie B., Thys W., Demeulemeester J., Borrenberghs D., Schrijvers R., Hofkens J., Bannert N. & Debyser Z.

60 Towards a DNA-based live-attenuated vaccine against the yellow fever virus and other flaviviruses
Dallmeier K., Mishra N., Kum D., Zmurko J. & Neyts J.

61 Bromodomain and extra-terminal (BET) proteins target Moloney murine leukemia virus integration to transcription start sites
De Rijck J., de Kogel C., Demeulemeester J., Vets S., Malani N., Bushman F., Busschots K., Husson S., Gijsbers R. & Debyser Z.

62 Ribavirin inhibits in vitro hepatitis E virus replication through depletion of cellular GTP pools and is moderately synergistic with interferon-alpha
Debing Y., Emerson S., Wang Y., Pan Q., Balzarini J., Dallmeier K. & Neyts J.

63 Active surveillance of swine influenza virus: potential role of porcine oral fluid samples
Decorte I., De Regge N. & Caij B.

64 Study of the mechanism by which a novel PI4KIII inhibitor and enviroxime inhibit HCV replication
Delang L., Andrews M., Leyssen P. & Neyts J.

65 2-Hydroxyisoquinoline-1,3(2H,4H)-diones (HIDs), novel inhibitors of HIV integrase with a high barrier to resistance
Demeulemeester J., Desimmie A., Suchaud V., Taltynov O., Billamboz M., Lion C., Bailly F., Strelkov S., Debyser Z., Cotelle P. & Christ F.

66 Feline herpesvirus ocular disease developing an antiviral ophthalmic solution for cats
Goris N., Auwerx J., Kiss E., Villers J., Billiet A., Hansen P. & Neyts J.

67 Comparison HIV-1 envelope DNA vaccine candidates within different animal models:

guinea pigs, rabbits and cynomolgus macaques
Heyndrickx L., Borggren M., Vinner L., Andresen B., Grevstad B., Repits J. et al.

68 Inhibition of Chikungunya virus replication by T-705 (favipiravir) and identification of resistance associated mutations in the RNA-dependent RNA polymerase
Jochmans D., Segura Guerrero N., Delang L., Pastorino B., Querat G., Dallmeier K., Bello F., Tas A., Snijder E., de Lamballerie X., Martina B., van Hemert M., Leyssen P. & Neyts J.

69 Discovery and development of a novel class of highly potent pan-serotype inhibitors of dengue virus replication that target NS4b
Kaptein S., Dallmeier K., Bardiot D., Carlens G., Koukni M., Smets W., Volny-Luraghi A., Chaltin P., Marchand A. & Neyts J.

70 Synergistic activation of HIV-1 expression by compounds targeting the positive transcription elongation factor b (P-TEFb) and by inducers of the NF-KB signaling pathway
Darcis G., Kula A., Bouchat S., Kabeya K., Delacourt N., Vanhulle C., Gatot J., Vaira D., Burny A., Herbein G., Clumeck N., Rohr O., Moutschen M., Rouzioux C., De Wit S. & Van Lint C.

71 A Michael acceptor inhibitor of the 3C protease with broad spectrum anti rhinoviral activity
Lacroix C., George S., Franco D., Leyssen P., Hilgenfeld R. & Neyts J.

72 Preventing the spread of foot-and-mouth disease virus with antiviral drugs
Lefebvre D., De Vleeschauwer A., Willems T., Rosseel T., Van Borm S., Goris N., Murao L., Kiss E., Kollanur D., Billiet A., Swinnen J., Neyts J. & De Clercq K.

73 Bovine herpesvirus 4 glycoprotein L is a major target of antibody neutralization
Lété C., Machiels B., Vanderplasschen A. & Gillet L.

74 Rotavirus genotype distribution in Belgium: continued high prevalence of G2, seven years after vaccine introduction
Matthijnssens J., Zeller M., Heylen E., Neels P., De Coster S. & Van Ranst M.

75 Eradication of persistent bovine viral diarrhea infection in cell culture by antiviral treatment: how to get ahead of the viral evasion strategy
Paeshuyse J., Puerstinger D. & Neyts J.

76 A new method to deliver an attenuated vaccine against bovine leukemia virus in herds
RamaRao G., Rodriguez S., Rodriguez S., Gutierrez G., Debrogniez A., Gillet N., Trono K. & Willems L.

77 Use of Staby® technology for development and production of DNA vaccines free of antibiotic resistance gene
Reschner A., Scohy S., Vandermeulen G., Daukandt M., Jacques C., Michel B., Nauwynck H., Xhonneux F., Préat V., Vanderplasschen A. & Szpirer C.

78 2'-C-methylcytidine efficiently prevents norovirus-induced diarrhea and mortality in a mouse model
Rocha-Pereira J., Jochmans D., Debing Y., Verbeken E., Nascimento M. & Neyts J.

79 SHe's a novel target for HRSV vaccination
Schepens B., Vande Ginste L., De Baets S., Sedyen K., Rossey I., Bogaert P., Gilbert B., Piedra P., Fiers W. & Saelens X.

80 Treatment with anti-rabies VHH prevents or delays disease and mortality in mice depending on the timing of treatment
Terryn S., Francart A., Lamoral S., Hultberg A., Vanlandschoot P., Rommelaere H., Wittelsberger A., Kalai M. & Van Gucht S.

81 CD4 down-modulating activity of cyclotriazadisulfonamide (CADA) analogs correlates with their anti-HIV potency
Van Puyenbroeck V., Schols D., Bell T. & Vermeire K.

82 Progress in the field of microbicides
Vanham G. & Ariën K.

83 Resistance of HCV replication to statins is conferred by cellular changes
Delang L., Verpaalen B. & Neyts J.

84 Targeting the dengue virus envelope protein by Escherichia Coli K5 derivatives to inhibit the infection of microvascular endothelial cells
Vervaeke P., Noppen S., Schols D., Oreste P. & Liekens S.

85 Redistribution of HIV-based viral vectors for safer gene therapy
Vets S., Brendel C., Grez M., Bushman F., Debyser Z. & Gijsbers R.

86 Antiviral agents to control classical swine fever epidemics: an epidemiologic and economic modeling study in a densely populated livestock area in The Netherlands
Vrancken R., Backer J., Neyts J. & Nesya G.

87 Effect of biocides on murine norovirus and feline calicivirus, surrogates of human norovirus
Zonta W., Mauroy A. & Thiry E.

Abstracts

1.

The Tax protein and the minichromosome maintenance protein complex MCM2-7 affect cell replication and viral transcription

Pierre-Yves Barez [1,2], Alexandre Carpentier [1,2], Mathieu Boxus [1,2] and Luc Willems [1,2*]

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The Tax oncoprotein plays a key role in the mechanisms of transformation, viral persistence and pathogenicity. Recently, we showed that Tax interacts with the minichromosome maintenance MCM2-7 helicase and binds to origins of DNA replication (Boxus et al, 2012 Blood 119:151). In fact, Tax modulates the spatiotemporal program of origin activation during the S phase of cell cycle. By this mechanism, Tax accelerates S phase progression through early firing of late replication origins. By interacting with the 5' LTR, the MCM2-7 complex also modulates Tax transactivation. Together, our data thus demonstrates that interaction between Tax and MCM2-7 modulates reprogramming of replication origins as well as viral transcription.

2.

The leader protein of Theiler's Murine Encephalomyelitis Virus inhibits stress granule assembly by blocking PKR activation.

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The DA strain of Theiler's Murine Encephalomyelitis Virus (TMEV) is a neurotropic picornavirus able to persist in the host central nervous system despite a strong immune response. This persistance leads to demyelinating lesions reminiscent of those found in multiple sclerosis (1). The virus' main weapon in counteracting the host immune system is the leader (or L) protein. It is shown to inhibit type I interferon and chemokine production (2), nuclear mRNA export (3), and recently, we showed that it inhibits stress granule assembly (4).

Stress granules are cytoplasmic aggregates of pre-initiation complexes of translation appearing in cells exposed to different types of stress (viral infection, oxidative stress, reticulum stress, heat shock, etc) (5). They trap mRNA and translation factors, and thereby slow down protein synthesis to reduce stress-induced protein misfolding.

We showed that stress granules appear in infected cells when the L protein is mutationally inactivated. Moreover, ectopic expression of the wild-type L protein was sufficient to inhibit oxidative stress-induced and reticulum-stress induced stress granule assembly (4).

Stress granule assembly is triggered by eIF2α phosphorylation (5). Four kinases are known to phosphorylate eIF2α: HRI, GCN2, PERK and PKR (activated by double-stranded RNA). We observed that infection-induced stress granule assembly correlates with PKR activation and eIF2α phosphorylation. Pharmacological inhibition of PKR activity, or knock down of PKR expression was sufficient to block eIF2α phosphorylation and stress granule assembly in infected cells. These results demonstrate that the wild-type L protein inhibits stress granule assembly by blocking PKR activation. The role of stress granules in the cellular defense against TMEV infection is unclear, however knock down of PKR expression dramatically increases replication of L-mutant viruses. This shows the importance of PKR inhibition for the virus.

We currently investigate the mechanism by which the L protein inhibits PKR activation.

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3.

Post-translational modification of the Myxoma virus chemokine-binding protein M-T7 by a virally encoded α-2,3-sialyltransferase.

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Myxoma virus is a pathogenic Poxvirus that induces a lethal disease called myxomatosis in european rabbits. It is one of the very rare viruses that encodes an α-2,3-sialyltransferase that transfers sialic acid to glycoproteins and glycolipids. Very little information is available about the role played by this glycosyltransferase in the pathogenesis of the infection. Previous experiments showed that the enzyme, encoded by the M138L gene, is not essential for virus replication *in vitro* but is important in the *in vivo* pathogenesis of myxomatosis. The objective of this study is the identification of the viral and cellular proteins modified by the α-2,3-sialyltransferase. A two-dimensional differential gel electrophoresis revealed that a target of the enzyme is the viral chemokine-binding protein M-T7. This information was confirmed by western blots. Moreover, a mass spectrometry glycan analysis of purified M-T7 proteins revealed precisely the nature of the modifications introduced by the M138L gene product. As M-T7 is a known important virulence factor of the virus, the difference in M-T7 sialylation could therefore be responsible of the *in vivo* attenuation observed during the infection with the M138L knockout virus. In the future, these results could help us to better understand the pathogeny of myxomatosis in european rabbits. Moreover, they could also help us to decipher the importance of glycans in host-pathogens interactions.

4.

Downregulation of several cell surface markers in equine herpesvirus 1 (EHV1) infected equine mesenchymal stem cells is dependent on the expression of the viral protein pUL56

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Equine herpesvirus type 1 (EHV1) is a ubiquitous alphaherpesvirus that will infect most horses during their life time, sometimes resulting in serious clinical symptoms with a considerable negative impact on horse welfare and economics. Clinical manifestations include respiratory disease, abortion, death of full term newborn foals and the emerging equine herpes myeloencephalopathy. During EHV1 pathogenesis, the virus is able to infect several cell types and tissues such as the respiratory epithelium and underlying connective tissues (primary replication) as well as the endothelium of the pregnant uterus and the central nervous system (secondary replication). Primary replication may be followed by subsequent cell-associated viremia, which results in systemic spread of the virus. The identity of infected carrier cells in the blood still remains a matter of debate. Recently, most of the infected cells in the blood were found to be positive for the cell surface marker CD172a (SIRPa) (SHPS1) (Gryspeerdt et al., 2012). Interestingly, mesenchymal stem cells (MSC), which can also be found in the blood, are CD172a positive in humans (Vogel et al., 2003). However, it is not known if MSC of horses, which are being used therapeutically in various clinical settings, are positive for CD172a expression (Fortier et al., 2011).This led us to investigate whether equine MSC, isolated from the peripheral blood, express CD172a and if so, whether they are susceptible to EHV1 infection.

Equine peripheral blood-derived MSC were positive for CD172a and susceptible to EHV1 infection. Interestingly, infection with EHV1 resulted in a significant downregulation of the immunophenotypical cell surface markers CD29 (B1-integrin), CD105 (endoglin) and MHCI. Expression levels of CD172a, CD44 and CD90 remained unaltered. EHV1-mediated downregulation of cell surface MHCI has been described in other cell types and has recently been shown to depend on the viral pUL56 protein (Ma et al., 2012). We found that EHV1-mediated MHCI downregulation in equine MSC is also pUL56-dependent. Importantly, and not reported before, downregulation of CD29 and CD105 was also pUL56-dependent.

In conclusion, we found that equine PBD-MSC are susceptible to EHV1 infection, and that EHV1 infection results in a marked and pUL56-dependent downregulation of CD29, CD105 and MHCI. Our data indicate (i) a potential role for equine PBD-MSC during EHV1 infection/viremia, (ii) that pUL56 may target specific (clusters of) cellular proteins for downregulation, and (iii) that EHV1 infection may complicate correct identification and restrict therapeutic use of equine MSC.

5.

Characterization of messenger RNA termini in Schmallenberg virus and related Simbuviruses

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Schmallenberg virus (SBV) is an emerging arthropod-borne virus in Europe. After its identification in 2011 September (Hoffmann *et al.*, 2012), SBV spread rapidly in several European countries. Although its relatively innocuous clinical signs in adult ruminants, SBV causes abortion, stillbirth and congenital malformations in newborn ruminants. Genetic analysis revealed that SBV belongs to the genus *Orthobunyavirus* within the *Bunyaviridae* family and is related to the Simbu serogroup viruses. Two studies have analysed the genetic background of SBV suggesting that SBV is included in reassortment events together with Sathuperi virus (STAV) and Shamonda virus (SHAV). The genome of the genus *Orthobunyavirus* comprising small (S), medium (M) and large (L) segments encodes six proteins. Whilst the genomic and antigenomic RNAs of each segment are identical in size, mRNAs differ in relation to their corresponding antigenomic RNAs at both ends.
By using different RACE-PCR (Rapid Amplification of cDNA Ends) strategies, SBV RNAs termini were characterized, and the mechanisms occuring in mRNA initiation and termination were investigated. 5' RACE analysis realized both *in vivo* and *in vitro* identified a cap-snatching mechanism used by segmented negative RNA viruses to initiate the RNA transcription. Presence of extraneous nucleotides between host RNA leaders and the viral termini fits with the previously described prime-and-realign theory (Garcin *et al.*, 1995). We used 3' RACE analysis to identify mRNA transcription termination. This investigation revealed common features for SBV and related Simbuviruses (SHAV and SATV). However, different patterns were observed for the three segments termini of a same virus. Precisely, mechanism that occurs on S segment seems to be conserved within the Simbu serogroup. Termini of S mRNA were mapped between a structurally conserved hairpin and a putative transcription termination signal. Terminations of M segment were distributed and L segment showed a 'runoff' transcription.

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6.

The ORF7-encoded Accessory Protein 7a of Feline Infectious Peritonitis Virus as a Counteragent against Interferon-alpha Induced Antiviral Response

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Abstract

The type I interferon (IFN)-mediated immune response is the first line of antiviral defence. Coronaviruses, like many other viruses, have evolved mechanisms to evade this innate response, ensuring their survival. Several coronavirus accessory genes play a central role in these pathways but for feline coronaviruses this was never studied. As it was demonstrated before that open reading frame (ORF) 7 is essential for efficient replication *in vitro* and virulence *in vivo* of feline infectious peritonitis virus (FIPV), the role of this ORF in the evasion from the IFN-α antiviral response was investigated. Deletion of ORF7 from FIPV 79-1146 rendered the virus more susceptible for IFN-α treatment. Given that ORF7 encodes for two proteins 7a and 7b, it was further explored which of these proteins are active in this mechanism. Providing 7a protein in trans rescued the mutant FIPV-Δ7 from IFN sensitivity, which was not achieved by addition of 7b protein. This result indicates that FIPV 7a protein is a type I IFN antagonist and attributes to the protection of the virus from the antiviral state induced by IFN.

7.

Integrase interface mutant reveals importance of transportin-SR2-integrase interaction in HIV nuclear import

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Background
Transportin-SR2 (TRN-SR2) is a human karyopherin encoded by the *TNPO3* gene. We identified TRN-SR2 as a binding partner of HIV-1 integrase (IN) and validated TRN-SR2 as an important cellular cofactor for the nuclear import of HIV-1. However, the question still remained whether the direct interaction between TRN-SR2 and IN mediates the nuclear import of HIV.
Materials and methods
By peptide screening we analyzed the interaction between TRN-SR2 and IN in molecular detail. Protein-protein interactions were verified by co-IP and AlphaScreen. We engineered site specific mutants in the C-terminal domain of HIV-1 IN that display reduced interaction with TRN-SR2 and studied their role in HIV replication using Q-PCR and fluorescence microscopy.
Results
Using the AlphaScreen protein-protein interaction assay we have been able to pinpoint the interacting hot spots in IN to R262/R263/K264 and K266/R269 in the IN C-terminal domain. We also identified a secondary interaction surface involving residues F185/K186/R187 and K188 in the catalytic core domain. Next, we introduced mutations at these positions in the C-terminal domain in the virus to corroborate the biological relevance of the interaction. Several mutations in the C-terminal domain of HIV IN inhibit the IN/TRN-SR2 interaction and render the virus replication deficient Some mutants affect reverse transcription (RT) compromising analysis of HIV nuclear import. All mutants also affected integrase activity. Still, one mutant, $IN^{R263A/K264A}$, retained RT activity but displayed a specific block at the level of nuclear import as measured by Q-PCR and fluorescence microscopy. Although this mutant was defective for integration, no increase in 2-LTR circles was detected. Moreover, $IN^{R263A/K264A}$ showed reduced nuclear import with an eGFP-IN labeled HIV.
Conclusion
The $IN^{R263A/K264A}$ mutation in the C-terminal domain of HIV-1 integrase, that reduces the interaction with TRN-SR2, specifically blocks HIV replication at the stage of nuclear import, corroborating the importance of this direct protein-protein interaction in HIV nuclear import.

8.

"STUDY OF THE VIRULENCE OF SEROTYPES 4 AND 9 OF THE ORBIVIRUS AFRICAN HORSE SICKNESS VIRUS IN TWO MOUSE MODELS."

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African horse sickness (AHSV) is an infectious disease caused by a double stranded positive RNA virus which belongs to the family *Reoviridae*, genus *Orbivirus*. The virus has nine serotypes and is transmitted by a culicoides biting midge, principally *Culicoides imicola*. African horse sickness causes severe morbidity and mortality up to 95 % in horses with severe economic losses. The establishment of an experimental model is needed for the investigation of the pathogenesis of this infection.
Two mouse models, interferon-α receptor knock-out mice (A129 KO or IFNAR -/-) and immunocompetent mice (A129 WT), were tested. The viruses used for mice inoculations belonged to the two serotypes which caused epidemics in Europe, serotypes 4 and 9. The virus was inoculated by subcutaneous (SC) route and/or by intra-nasal (IN) route. Whole blood samples were taken from each mouse at regular intervals. The organs (liver, spleen, kidney, lung and brain) were taken at the end of the experiment or when the most affected mice were euthanized. All these samples were tested by a qRT-PCR targeting AHSV genome segment 7.
Both serotypes of AHSV were detected by qRT-PCR until three weeks post-infection in blood of IFNAR -/- mice and A129 WT mice infected by SC route. Serotype 4 shows a higher peak of viremia than serotype 9. The peak of viremia was measured between day 2 and day 4 post-infection. These results demonstrate the potential of the immunodeficient mouse model for both clinical and biological features.
The setting up of this mouse model has developed a tool for efficient *in vivo* study to characterize the *in vivo* virulence of this virus, to monitor the evolution of viral populations during *in vivo* replication cycles and to test the competence or vectorial capacity of indigenous Culicoides.
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9.

Establishment of feline intestinal epithelial cell cultures for the propagation and study of feline enteric coronaviruses

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Feline infectious peritonitis (FIP) is the most feared infectious cause of death in cats, induced by feline infectious peritonitis virus (FIPV). This coronavirus is a virulent mutant of the harmless, ubiquitous feline enteric coronavirus (FECV). To date, feline coronavirus (FCoV) research has been hampered by the lack of susceptible cell lines for the propagation of serotype I FCoVs. In this study, long-term feline intestinal epithelial cell cultures were established from primary ileocytes and colonocytes by simian virus 40 (SV40) T-antigen- and human Telomerase Reverse Transcriptase (hTERT)-induced immortalization. Subsequently, these cultures were evaluated for their usability in FCoV research. Firstly, the replication capacity of the serotype II strains WSU 79-1683 and WSU 79-1146 was studied in the continuous cultures as was done for the primary cultures. In accordance with the results obtained in primary cultures, FCoV WSU 79-1683 still replicated significantly more efficient compared to FCoV WSU 79-1146 in both continuous cultures. In addition, the cultures were inoculated with faecal suspensions from healthy cats and with faecal or tissue suspensions from FIP cats. The cultures were susceptible to infection with different serotype I enteric strains and two of these strains were further propagated. No infection was seen in cultures inoculated with FIPV tissue homogenates. In conclusion, a new reliable model for FCoV investigation and growth of enteric field strains was established. In contrast to FIPV strains, FECVs showed a clear tropism for intestinal epithelial cells, giving an explanation for the observation that FECV is the main pathotype circulating among cats.

10.

Impact of latent Murid herpesvirus 4 infection on the development of an anti-pneumovirus vaccine-induced respiratory immunopathology.

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Gammaherpesviruses are lymphotropic viruses which persist lifelong in their host by establishing and maintaining a lifelong latent infection. Murid herpesvirus 4 (MuHV-4) is a natural pathogen of wild murid rodents and provides a valuable gammaherpesvirus small animal model. Recently, it has been demonstrated that this virus modulates the immune system of its host and offers symbiotic protection against heterologous infections and allergic disorders. The human respiratory syncytial virus (hRSV) is a ubiquitous pathogen which induces lower respiratory infections and Th2-skewed immunopathologies such as the particular hRSV vaccine-enhanced disease. Interestingly, all these events have been successfully reproduced in the mouse by the use of the Pneumonia virus of mice (PVM). In a homologous host-pathogen model, we decided therefore to explore the impact of latent MuHV-4 infection on the development of the respiratory Th2-skewed immunopathology which is induced by a formalin-inactivated PVM vaccine and a subsequent respiratory PVM infection. Our results demonstrated that MuHV-4 infection, both before or after the PVM vaccination, significantly protected the host from the development of the pathological Th2-biased immune response. Notably, we observed lower levels of total leukocytes, eosinophils, IL-4, IL-5 and IFN-γ in broncho-alveolar lavages of PVM-challenged MuHV-4 infected mice than in mock infected mice. Interestingly, while the MuHV-4 infection blocked the Th2-skewed immunopathology, it dit not affect the inhibition of PVM replication conferred by the vaccine. In conclusion, this study has proved that MuHV-4 infection induces profound immunomodulations which confer protection to the host against a vaccine-induced respiratory immune disorder. In the future, we would like to discover the underlying mechanisms of these immunomodulations, both at cellular and molecular levels.

11

Characterization of L* of different Theiler's virus strains

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The DA strain of Theiler's virus has a striking ability to evade the immune response of the host and to cause chronic infections of the central nervous system. Viral persistence and the accompanying immune response are responsible for a demyelinating disease. The L* protein encoded by Theiler's virus is a non-structural protein that is required for the establishment of persistent infections. It facilitates the infection of macrophages and of other cells when these cells are pre-treated with interferon (IFN). Frédéric Sorgeloos, in our lab, showed that L* inhibited the activity of RNase L, one of the best-characterized effector of the IFN pathway. In cells that were primed with IFN, viral replication activates RNase L which, in turn, degrades both viral and cellular RNA, thereby triggering apoptosis and limiting viral spread.
L* acts directly interacts with RNase L to inhibit enzyme activation. Interestingly, L* acts in a species-specific fashion as it inhibits mouse (and to a lesser extent rat) RNase L but not human, bovine, avian or any other tested RNAse L.

We took advantage of this species-specific activity of L* to answer open questions about the origin and phenotype of Theiler's virus strains. One of these question is related to the origin of the Vilyiusk strain of Theiler's virus.
Vilyuisk virus was isolated in Siberia, in the 1960's, after an epidemics of endemic encephalitis. The virus was recovered from newborn mice that were inoculated with cerebro-spinal fluid from Siberian patients who died from this encephalitis. It is thus unclear whether this virus derives from the human samples or from a contaminant of the inoculated mice. In this context, we tested whether L* of Vilyuisk virus exhibits specificity to the human or the murine RNase L. We cloned the L* coding sequence of Vilyuisk virus from a synthetic plasmid and expressed this protein using plasmidic and lentiviral constructs. Co-immunoprecipitation experiments and functional assays showed that L* of Vilyuisk interacts with and inhibits the murine but not the human RNase L. Our data suggest that Vilyuisk virus would therefore rather be of murine origin. Yet, phylogenetic analyses of capsid proteins suggest that this virus is distantly related to the cluster of mouse Theiloviruses. The link between this virus and Vilyuisk encephalitis will be discussed.

12.

Evaluation of West Nile virus (WNV) NS3Thr249Pro mutation as a virulence marker in a SPF Chicken model and in Carrion crows (*Corvus corone*)

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West Nile virus (WNV) has become a wide-spread arbovirus in Europe and the Mediterranean Basin countries. This emerging zoonotic disease disseminated 14 years ago in North America where its impact on animal and public health has been considerable. Particularly, American crows were highly susceptible to fatal infection with the original American topotype strain NY99 whose virulence was linked to the presence of a proline residue at position 249 of the viral NS3 helicase. However, the $NS3_{249Pro}$ genotype has arisen recently in the Western Mediterranean region but did not always correlate with an enhanced virulence for birds. This discrepancy prompted us to evaluate whether this substitution is responsible for the pathogenicity of the European WNV strain Is98 that had elicited high rates of avian deaths in 1998 in Israel and is characterized by an $NS3_{249Pro}$ genotype.

We therefore used two clones derived from the Is98 strain, (1) a wild type (WT) clone characterized by a $NS3_{249Pro}$ genotype and (2) a mutant clone characterized by a $NS3_{249Thr}$ genotype, which we inoculated to wild-caught WNV-sensitive Carrion crows and to a WNV infection avian model, namely, SPF chickens aged one day. Clinical and serological follow-up as well as viral RNA load monitoring in sera, oral swabs, feathers and inner organs by an in-house rRT-PCR specific to NS2a viral protein was performed on the experimentally inoculated birds.

When inoculated to the SPF chicks, the WT clone elicited higher mortality rates than the mutant one (50% vs. 16%), as well as a higher and longer lasting viremia (7 days vs. 5 days). Similarly, all the crows inoculated with the WT clone died, whereas the mortality rate in the mutant clone group amounted to 17%. This low mortality rate in the mutant clone cohort correlated with the absence of any clinical signs of disease in addition to a viremia, viral RNA loads in the inner organs, and neutralizing antibodies titers that were below the detection limits of the assays. Conversely, WT clone-inoculated crows were viremic all over the infection period, developed a systemic infection, and had sero-converted by 6 days post-infection. Futhermore, viral loads were higher in the feathers and oral swabs of the crows inoculated with the WT clone than with the mutant one.

Altogether, the data we present show that two identical infectious clones that are derived from IS98 WNV strain and only differ in NS3 249^{th} residue exhibited differential pathogenicities in an avian model for WNV infection (i.e.: the SPF chick aged 1 day) as well as in a sensitive natural avian host (i.e.: the Carrion crow) since the $NS3_{249Pro}$ genotype induced higher mortality rates and viral RNA loads than the $NS3_{249Thr}$ genotype in both bird species. Thus, we could demonstrate that the Thr→Pro substitution is truly a determinant of virulence of IS98 strain, as it was once demonstrated for NY99 strain by Brault and co-workers (2007). Moreover, and with regards to the complex Western Mediterranean epidemiological situation,

these findings bring evidence that other residues that differ between isolates might also modulate WNV virulence.

13.

Massive Depletion of Bovine Leukemia Virus Proviral Clones Located in Genomic Transcriptionally Active Sites During Primary Infection

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Deltaretroviruses such as human T-lymphotropic virus type 1 (HTLV-1) and bovine leukemia virus (BLV) induce a persistent infection generally asymptomatic but can also lead to leukemia or lymphoma. These viruses replicate by infecting new lymphocytes (i.e. the infectious cycle) or via clonal expansion of the infected cells (mitotic cycle). The relative importance of these two cycles in viral replication varies during infection. The majority of infected clones are created early before the onset of an efficient immune response. Later on, the main replication route is mitotic expansion of pre-existing infected clones. Due to the paucity of available samples and for ethical reasons, only scarce data is available on early infection by HTLV-1. Therefore, we addressed this question in a comparative BLV model. We used high-throughput sequencing to map and quantify the insertion sites of the provirus in order to monitor the clonality of the BLV-infected cells population (i.e. the number of distinct clones and abundance of each clone). We found that BLV propagation shifts from cell neoinfection to clonal proliferation in about 2 months from inoculation. Initially, BLV proviral integration significantly favors transcribed regions of the genome. Negative selection then eliminates 97% of the clones detected at seroconversion and disfavors BLV-infected cells carrying a provirus located close to a promoter or a gene. Nevertheless, among the surviving proviruses, clone abundance positively correlates with proximity of the provirus to a transcribed region. Two opposite forces thus operate during primary infection and dictate the fate of long term clonal composition: (1) initial integration inside genes or promoters and (2) host negative selection disfavoring proviruses located next to transcribed regions. The result of this initial response will contribute to the proviral load set point value as clonal abundance will benefit from carrying a provirus in transcribed regions.

14.

A novel mechanism of alphaherpesvirus immune evasion: the pseudorabies virus glycoprotein gD interferes with Natural Killer cell-mediated lysis of infected cells

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Natural Killer (NK) cells are key players in the innate response against viral infections, and are important in preventing alphaherpesviruses from causing life-threatening encephalitis (Etzioni, 2005, J. Pediatr. and Almerigogna, 2011, Int. J. Immunopathol Pharmacol). Still, alphaherpesviruses evade the innate immune system sufficiently to spread and establish latency. NK-mediated killing of target cells is regulated by a complex sum of signals received through activating and inhibiting receptors on the NK cell surface. Viral glycoproteins, expressed on the cell membrane of infected cells, may influence this balance by affecting, directly or indirectly, the efficiency of binding of activating and/or inhibitory receptors on the NK cell surface with the infected cell.

Here, we report that porcine SK cells infected with a porcine alphaherpesvirus pseudorabies virus (PRV) variant that lacks the glycoprotein gD are more susceptible to lysis by primary porcine NK cells than cells infected with wild type PRV, suggesting that gD has an inhibitory effect on NK cell activity. Transfection studies confirmed that expression of PRV gD reduces susceptibility of cells towards NK-mediated lysis. PRV gD has been reported to interact with immunoglobulin-like superfamily members nectin1, nectin2 and poliovirus receptor (PVR)(Geraghty et al., 1998, Science, Warner et al., 1998, Virology). Interestingly, the latter two are known ligands for the activating NK receptor DNAM1 (Bottino et al., 2003, J. Exp. Med.). To assess whether expression of PRV gD reduces availability of nectin2 and PVR on the cell surface, thereby reducing binding of DNAM1 to infected/transfected cells, 293T cells were used. First, the suppressive effect of PRV gD towards NK-mediated lysis was confirmed in 293T cells. Next, flowcytometric analysis showed that PRV gD indeed results in an almost complete reduction of nectin2 availability on the cell membrane of 293T cells, and a less pronounced reduction in availability of PVR. Importantly, expression of PRV gD suppressed binding of recombinant DNAM1 to the 293T cell surface. In addition, both in 293T cells and SK cells, DNAM1 blocking assays confirmed that expression of gD reduces sensitivity of cells towards DNAM1-dependent NK-mediated lysis.

In conclusion, we show that expression of PRV gD lowers susceptibility of cells towards NK-mediated lysis through a reduced cell surface availability of DNAM1 ligands nectin2 and PVR, representing a novel mechanism of herpesvirus immune evasion.

15.

Human but not mouse hepatocytes respond to interferon-lambda *in vivo*

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The type III interferon (IFN) receptor, IL-28Rα, is preferentially expressed by epithelial cells. Human hepatocytes also express IL-28Rα and respond to IFN-lambda (IFN-λ). In contrast, the IFN-λ response of the mouse liver is very weak and IL-28Rα expression is hardly detectable in this organ. Here we investigated whether human and mouse hepatocytes truly differ in responsiveness to IFN-λ or whether this discrepancy is due to technical issues. When monitoring expression of the IFN-responsive Mx genes by immunohistofluorescence, we observed that the IFN-λ response in mouse livers was restricted to cholangiocytes which form the bile ducts and that mouse hepatocytes were indeed not responsive to IFN-λ. With the help of chimeric mice containing transplanted human hepatocytes, we show that hepatocytes of human origin readily responded to IFN-λ in a murine environment. Thus, our data demonstrate that human but not mouse hepatocytes are responsive to IFN-λ. The non-responsiveness is an intrinsic property of mouse hepatocytes and is not due to the mouse liver micro-environment.

16.

RABBIT COLONY INFECTED WITH A BOVINE G6P[11] ROTAVIRUS STRAIN

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Group A rotaviruses (RVAs) are the main etiological agent of infantile diarrhea. Only a few lapine RVA strains have been isolated and (partially) characterized to date. The most common G/P-genotype combinations in rabbits are G3P[14] and G3P[22]. There are only three completely characterized lapine(-like) RVA strains known today: RVA/Rabbit-tc/ITA/30-96/1996/G3P[14] and RVA/Human-wt/BEL/B4106/2000/G3P[14], both possessing the G3-P[14]-I2-R2-C2-M3-A9-N2-T6-E5-H3 constellation and RVA/Rabbit-tc/CHN/N5/1992/ G3P[14] possessing a G3-P[14]-I17-R3-C3-M3-A9-N1-T1-E3-H2 constellation.
In a diagnostic laboratory for health monitoring of laboratory animals in the Netherlands, a RVA strain was isolated from the small intestine of a 9 weeks old rabbit from an infected animal colony. The RVA strain RVA/Rabbit-tc/NLD/K1130027/2011/G6P[11] was adapted to MA104 cells, and was shown to possess the typical bovine G6P[11] genotypes. In this study we sequenced and characterized the complete genome of this unusual strain. Phylogenetic analyses of all 11 gene segments revealed the following genotype constellation: G6-P[11]-I2-R2-C2-M2-A13-N2-T6-E2-H3. The VP1, VP2, VP3, VP6, NSP2 and NSP4 genes all belonged to genotype 2 which clustered more closely to bovine RVA strains than to lapine RVA strains. The NSP1 genotype A13 is typically associated with bovine RVAs, while the NSP3 genotype T6 and the NSP5 genotype H3 have been found in a wide variety of species. However, our strain clustered within bovine(-like) T6 and H3 subclusters. Overall, the data indicate that RVA strain K1130027 is most closely related to bovine-like RVA strains and most likely represents a direct interspecies transmission from a cow to a rabbit. Altogether, these findings indicate that a RVA strain with an entirely bovine genome constellation was able to infect and cause disease in a laboratory rabbit colony.

17.

Role of the RhoA signaling pathway in cytoskeletal rearrangements induced by the alphaherpesvirus US3 kinase

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Alphaherpesviruses constitute the largest subfamily of the herpesvirus family. In humans, they can cause a variety of disease symptoms such as cold sores and genital lesions (herpes simplex virus), and chickenpox and shingles (varicella zoster virus). The closely related porcine alphaherpesvirus pseudorabies virus (PRV) is often used as a model organism to study general aspects of alphaherpesvirus biology (Pomeranz et al., 2005).

The US3 kinase is a conserved alphaherpesviral serine/threonine kinase that, amongst other functions, causes rearrangements in the actin cytoskeleton, consisting of actin stress fiber disassembly and the formation of cell projections, which are associated with increased intercellular virus spread (Favoreel et al., 2005). The mechanism of US3-mediated actin rearrangements is poorly understood but depends on the activity of p21-activated kinases (PAKs) (Van den Broeke et al., 2009). PAKs are negatively influenced by the RhoA pathway, which has opposite effects on the cytoskeleton (i.e. formation of actin stress fibers).

In this report, we investigated whether (inactivation of) the RhoA signaling pathway is involved in US3-mediated actin rearrangements. An important RhoA inactivation mechanism relies on phosphorylation of RhoA on position S188, which results in RhoA sequestration in the cytoplasm (Ellerbroek et al., 2003). Interestingly, and pointing towards a potential role for RhoA phosphorylation at S188 in the US3 phenotype, cotransfection of porcine ST cells with US3 and non-phosphorylatable RhoA (S188A) led to a decrease in cells displaying the US3-phenotype compared to co-transfection of US3 with WT RhoA. Additionally, cotransfection of US3 with dominant active (DA) mDia, a RhoA effector, interferes with the US3 cytoskeletal phenotype, suggesting that US3 may indeed interact in the RhoA signaling axis. Our research group has shown earlier that PAK activity is important for the US3 phenotype (Van den Broeke et al., 2009). PP2C family phosphatase POPX2 might constitute a link between PAK and RhoA/mDia as POPX2 is described to interact with mDia (Xie et al., 2008) as well as to inactivate PAK (Koh et al., 2002). To further investigate the role of this POPX2-dependent negative feedback loop between mDia and PAK, triple transfection experiments were performed. US3 was transfected along with DA mDia and POPX2, the latter either wild type (WT) or mutated to phosphatase-dead POPX2. Phosphatase-inactive POPX2 partially

reversed the suppressive effect of DA mDia on the US3-phenotype, while this was not the case using WT POPX2.

In summary, we suggest that US3 interferes with the RhoA pathway, possibly by affecting phosphorylation of RhoA at S188. Moreover, by interfering with the RhoA pathway, US3 enforces its effect on the PAK pathway by shutting down the RhoA/mDia/POPX2-mediated negative feedback loop to PAK.

18.

Characterization of an infectivity assay for the human parvovirus B19 based on the HepG2 hepatocarcinoma cell line

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Human parvovirus B19 virus is the most commonly responsible for mild disease including erythema infectiosum in childhood and arthropathy in young adult. As a consequence of the tropism of B19 for progenitors of erythroid cells, B19 infections can also cause more severe disease, such a transient aplastic crisis in patients suffering from chronic hemolytic disorders and fetal infection during pregnancy that can lead to spontaneous abortion, fetal hydrops, or fetal death. B19 infectivity assays have been developed in erythroid cell lines (UT7, K562) but they do not lead to the production of infectious viral progeny indicating that B19 viral cycle is not completed in these cells. We have developed an infectivity assay in the hepatocarcinoma cell line HepG2 *(Caillet-Fauquet et al, Transfusion 2004).* Briefly, subconfluent cells are infected at a low multiplicity of infection and 48 hours post-infection, the viral progeny in the cellular supernatant is measured by quantification of the viral DNA with quantitative B19 PCR tests. This viral progeny is infectious since successive runs of infection can be performed leading to productive amplifications of B19. The B19 amplification is prevented by preincubation of the virus with antibodies raised against the capsid VP proteins. Different genotype 1 B19 strains (WHO strain and blood-donnor units) multiply in HepG2 and up to 5 successive runs lead to an efficient production. The viral progeny of the fifth run was sequenced and found identical to the inoculum of the first run, demonstrating the genomic stability of the B19 produced in this infectivity test. The HepG2-based infectivity assay also allowed for the first time to define an infectious unit for B19, that corresponds to 0,1 international unit (IU) and 30 genome equivalents. This assay provides thus a very sensitive assay to detect B19 since its threshold is 0,1 IU while 10 IU are required to give a positive signal with the most sensitive PCR assay. This model is also a precious tool to study the viral cycle of B19, particularly the late steps of the cycle that remain largely unknown. This infectious model is currently under characterization in our laboratories as well as the relevance of the permissiveness of hepatocytes to B19 infections.

19.

Genome-wide shRNA screening identifies new host factors involved in HIV-1 mediated CD4 downmodulation.

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Downmodulation of the CD4 receptor expressed on T cells is one of the hallmarks of HIV infection and it is believed to confer to the virus a selective replicative advantage in vivo. HIV has evolved redundant mechanisms to remove the receptor from the cell surface and accelerate its degradation. This process is mainly mediated by two viral proteins: Vpu and Nef. Up to date, the mechanisms that lead to CD4 depletion from the surface of CD4+ T lymphocytes, the natural targets of HIV, are still poorly understood and only partially characterized. We are interested in the discovery of pathways and human proteins involved in the process. To pursue our aim, we first performed a functional screening on HeLa CD4+ cells expressing Nef as an inducer of CD4 downmodulation using a shRNA lentiviral delivery system targeting the whole human genome. The read-out was the rescue of CD4 expression despite the presence Nef: shRNA sequences enriched in the CD4-high cells compared to the CD4-low cells were identified and filtered via pathway analysis. After four different screens we obtained a final list of 75 genes ("hits"), which mainly encode for proteins involved in endocytic trafficking, trans-Golgi trafficking and lysosomal degradation pathways. The genome-wide approach is useful to perform a first selection of possible new factors, but for the confirmation and further selection of the hits we performed validation experiments in two consecutive rounds of experiments. First we transduced HeLa CD4+ cells with shRNA lentiviral vectors (Sigma Aldrich) for each hit separately, followed by Nef transduction. Second, the 22 genes confirmed in the first validation round were used for confirmation in a functionally more relevant setting involving transduction of SupT1 cells with the shRNA lentiviral vectors for each of the 22 selected hits and subsequent infection with HIV-1. The latter confirmed 4 final hits (GGA1, HRBL, DNM3 and SNX22) whose knock-down increased CD4 levels in HIV-infected cells compared to the negative control. The shRNA- mediated knock-down of the target genes in SupT1 cells was confirmed via qRT-PCR. Finally, we expressed the Vpu and Nef proteins separately via retroviral vectors in SupT1 cells expressing shRNA targeting one of the 4 final hits to evaluate which of the two viral proteins contributed to the rescue observed during HIV infection. For HRBL the rescue of CD4 levels is observed both for Nef and Vpu, while for SNX22 and GGA1 the rescue is observed only when CD4 is downmodulated by Vpu. Major effect of DNM3 knock-down on CD4 levels is observed only during HIV infection and not when only Nef or Vpr is expressed seperately. Our results confirm intrinsic differences between Nef and Vpr in the mechanism of CD4 downmodulation. Future efforts will characterize the mechanisms involved and assay the hits in primary CD4+ T lymphocytes.

20.

Abstract for Belgian Society of Virology (November 9th 2013) :

Title : « Generation of trans-encapsidated Theiler's virus replicons »
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In order to study cell-to-cell transmission modes and axonal transport of Theiler's virus, we generated trans-encapsidated replicons of this virus.

A replicon is a segment of viral genome which is able of autonomous replication in a cell but which cannot give rise to new infectious particles since it does not encode some critical components of the virion. In our replicons, the sequence coding for the viral capsid proteins was replaced by the sequence coding for GFP.

To allow encapsidation of the replicon in trans, the ORF encoding the capsid protein precursor was cloned in a lentiviral vector. 293T cells transduced with this construct constitutively express the capsid precursor. Trans-encapsidated replicons were produced by transfection of capsid-producing cells with replicon RNA. In picornaviruses, it has been shown that encapsidation was coupled with RNA replication. It was thus unclear whether capsid proteins provided in trans would faithfully package newly synthesized RNA strands produced in localized replication complexes.
FACS analysis and fluorescent microscopy confirmed that we obtained infectious particles (encapsidated replicons) that can perform a single infection cycle.
We showed that the propagation of our replicon in the producing cells occured only in the presence of the viral capsid proteins and that the replicon was able to process the polyprotein expressed from our lentiviral vector.
In addition, mutations introduced in the CRE (cis-acting replication) sequence of the capsid sequence prevented the emergence of recombinant infectious virus in our encapsidated replicon stocks.

These trans encapsidated replicons will be of a great interest for the study of the spread of the virus from cell to cell and for the dissection of the mechanism of the viral transport along axons.

21.

Murid herpesvirus 4 ORF63 is not essential but contributes to virus replication and latency establishment.

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The immune response to viral infections is determined by a complex interplay between the pathogen and the host. This is particularly true for persistent viruses such as gammaherpesviruses. While evasion of the adaptive immune response by these viruses is well described, less is known about how they evade innate immunity. Interestingly, a recent study showed that the protein encoded by the ORF63 gene of the Kaposi Sarcoma associated Herpesvirus (KSHV) could inhibit inflammasome activation *in vitro*. However, as this virus has no well-established *in vivo* infection model, the importance of this observation in natural conditions is unknown. In this work, we studied the homologous ORF63 protein of Murid herpesvirus-4 (MuHV-4). Firstly, we showed that this proteins shares potential structural folds with KSHV ORF63 suggesting that both proteins could have similar functions. Secondly, we showed that a lack of ORF63 was associated with severe deficits both *in vitro* and *in vivo*. Interestingly, analysis of host colonization revealed that this deficit was mainly associated with a defect during the viral lytic cycle in lung but did not appear to be due to reduced ability to establish latency. Indeed, while early latency loads were reduced in mice infected by the MuHV-4 ORF63 deficient strains in comparison with WT and revertant strains, these loads were similar at later time points. Altogether, our results showed that ORF63 is non-essential but however very important for the MuHV-4 lifecycle. In the future, these results should help us to decipher the role of ORF63 in the lifecycle of MuHV-4 and of gammaherpesviruses in general.

22.

LDV exacerbation of Fc receptor-mediated autoimmune blood diseases

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Lactate dehydrogenase-elevating virus (LDV) is a RNA single-stranded enveloped murine arterivirus infecting a not-so-well defined subset of macrophages. Although LDV does not usually induce any pathology in mice, the infection leads to the exacerbation of some autoimmune blood diseases mediated by macrophage activation.
The cellular receptors for immunoglobulin (FcRs) play an important role in the immune response, one of them being the regulation of phagocytosis. To date, four FcRs to IgG have been identified in mice: FcγRI, FcγRIIb, FcγRIII and FcγRIV, while FcαμR is the only receptor for IgM expressed by mice macrophages.

Our results indicate that on infection with LDV, the number of macrophages expressing Fc**γ**RI, FcγRIII and FcγRIV is increased. To better understand the contribution of these receptors in LDV-exacerbated anemia, we used 105-2H IgG1 or 34-3-C IgG2a anti-red blood cells (RBC) monoclonal antibodies (mAbs) in infected animals. 34-3C mAb binds to FcγRI, FcγRIII and FcγRIV with a high, low and moderate affinity respectively while 105-2H mAb binds only to FcγRIII with a low affinity. The administration of 105-2H mAbs triggered a moderate and transient anemia in WT mice but not in FcγRIII KO mice. The administration of a lesser dose of 34-3C IgG2a mAb in LDV-infected WT mice led to a more severe anemia both *in vivo* and *in vitro* while erythophagocytosis was not completely abolished in FcγRIII KO and FcγRIIb-III-IV KO mice.
We also showed that *in vitro* erythrophagocytosis of 34-3C opsonized-RBC by macrophages derived from infected mice is completely prevented in their FcγRI-IIb-III-IV KO counterpart. This suggests that the expression of FcRs is necessary for LDV exacerbation of autoimmune anemia, at least *in vitro*.
We further assessed the role of type I and type II interferons (IFN) in LDV-exacerbated anemia. On blocking IFNγ and IFNγR of infected IFN-$\alpha R^{0/0}$ mice, we observed a strong decrease in *in vitro* erythrophagocytosis by macrophages of both 105-2H and 34-3C opsonized-RBC suggesting an involvement of both type I and type II IFN in LDV-exacerbated anemia.
Since some autoimmune diseases may be mediated by IgM rather than IgG autoantibodies we investigate the involvement of Fcαμ receptor in the exacerbation of thrombocytopenia in LDV-infected mice. The administration of L1C43 or L1H31 IgM anti-platelets mAbs in WT mice triggered the development of a moderate thrombocytopenia as well as LDV infection alone. We also showed that antibody administration and LDV infection act synergistically to decrease the platelet count in WT animals but not in FcαμR KO mice.

In conclusion our results show that LDV-infection induces an increase in the number of macrophages expressing Fc receptors following infection. We also suggest that LDV infection plays an important role in exacerbation of anemia triggered by IgG2a mAbs through FcgRI and FcgRIII receptors. Furthermore the expression of FcRs seems to be necessary in LDV exacerbation of *in vitro* erythrophagocytosis, which is regulated by both type I and type II IFN. Our work also indicates that LDV infection increases the pathogenicity of anti-platelets IgM mAbs, which is mediated by FcαμR binding on activated-macrophages.

23.

A gammaherpesvirus uses alternative splicing to regulate its tropism and its sensitivity to neutralization.

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Human gammaherpesviruses are associated with the development of lymphomas and epithelial malignancies. The heterogeneity of these tumors reflects the ability of these viruses to route infection to different cell types at various stages of their lifecycle. While the Epstein Barr virus uses gp42 – human leukocyte antigen class II interaction as a switch of cell tropism, the molecular mechanism that orientates tropism of rhadinoviruses is still poorly defined. Here, we used bovine herpesvirus 4 (BoHV-4) to further elucidate how rhadinoviruses regulate their infectivity. In the absence of any gp42 homolog, BoHV-4 exploits the alternative splicing of its Bo10 gene to produce distinct viral populations that behave differently based on the originating cell. While epithelial cells produce virions with high levels of the accessory envelope protein gp180, encoded by a Bo10 spliced product, myeloid cells express reduced levels of gp180. As a consequence, virions grown in epithelial cells are hardly infectious for CD14+ circulating cells, but are relatively resistant to antibody neutralization due to the shielding property of gp180 for vulnerable entry epitopes. In contrast, myeloid virions readily infect CD14+ circulating cells but are easily neutralized. This molecular switch could therefore allow BoHV-4 to promote either, on the one hand, its dissemination into the organism, or, on the other hand, its transmission between hosts.

24.

Experimental evidence of reassortment in Simbuviruses related to Schmallenberg virus, an emerging arbovirus in Europe

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Background: In 2011, a novel virus, the Schmallenberg virus (SBV), has been detected in blood sample from adult cow showing a new disease characterized by high fever, anorexia, apathy, milk drop and diarrhoea. Since then, the virus has been detected in several domestic and wild ruminants all over Europe. Following transplacental infection of pregnant ruminants, SBV induces congenital defects referred to as arthrogryposis hydranencephaly syndrome in neonates. Phylogenetic analysis showed that SBV belongs to the genus *Orthobunyavirus,* within the family *Bunyaviridae* and is a member of the Simbu serogroup viruses. The segmented genome of SBV is composed of three negative single stranded RNA molecules, designated large (L), medium (M) and small (S) that altogether encodes 6 proteins. These RNA segments display a high mutation rate since the RNA dependent RNA polymerase is devoid of proof reading and 3'-5' exonuclease activities. SBV replication generates thus a large panel of mutants from an original infecting virus. Intramolecular recombination and reassortment are two ways to disseminate these mutations in a viral population. *Orthobunyavirus* reassortants have been described by phylogenetic analyses but few data are available concerning the genetic origin of SBV virus. Two viruses have been described to be related to SBV: (i) Shamonda virus (SHAV) containing S and L segments closely similar to the SBV respective segments; (ii) Sathuperi virus (SATV) whose M segment is close to the SBV M RNA. The aim of this study was to experimentally investigate both recombination and reassortment between these two Simbuvirus related to SBV.

Material and methods: SHAV and SATV were used to co-infect mammalian cells in two conditions of co-infection at low and high multiplicities of infection (M.O.I). A method was set up to generate, isolate and amplify clonal progeny viruses. Discriminative PCR were developed at both extremities (5' and 3') to determine the genetic background of the three segments in each progeny virions. Genomic characterization was completed with phenotypic assessment by using plaque size analyses, growth kinetics and stability of progeny virions over time.

Results: A total of 138 progeny virions were plaque purified and genotyped from the two different situations of co-infection. Reassortment events occurred at a high rate since reassortant frequencies were 14 % and 20 % of the viral progeny following the co-infections at low and high M.O.I respectively. The six potential reassortants were generated in both conditions. Among them, several SBV-like reassortants ($S^{SHAV}/M^{SATV}/L^{SHAV}$) were generated. In contrast no intramolecular recombination event has been identified among the progeny viruses. Plaque size analysis showed a statistical difference among the different types of reassortants. Analysis related to the comparison of the growth kinetics and the stability of the isolated progeny viruses are in progress.

Conclusion: This study brings new insights regarding the rise of SBV and more generally *Bunyaviridae* evolution. Moreover, the generation of different reassortants will allow exploring the relationship between viral genotype and phenotype. These findings

will be used to better understand how cooperative interactions among two related viruses contribute to viral fitness and evolution.

25.

Bovine noroviruses in Belgium: from a molecular and evolutionary perspective.

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Noroviruses are single stranded positive sense RNA viruses belonging to the *Caliciviridae* family and are considered as major etiological agents of acute non-bacterial gastroenteritis in humans. Genetically related viruses were also detected in stool samples from different animal species, in particular from cattle, logically raising the hypothesis of a zoonotic risk or a bovine reservoir. Noroviruses are distributed into different genogroups further divided into genotypes. All the bovine noroviruses sequences cluster into genogroup III wherein 2 genotypes are described.

Here are reported the results of molecular and genetic studies designed to resolve the zoonosis hypothesis in the Belgian epidemiological context and performed along an 8 years-long period.

No human norovirus sequence was detected in calf stools and phylogenetic relationships showed that genotype 2 strains were the more prevalent. It raises hypothesis on their better host adaptation and reinforces the low zoonotic risk currently associated to bovine noroviruses, as the attachment factors identified so far for genotype 2 bovine noroviruses, the α-gal epitope, is not expressed on human cells.

Recombinant strains were detected during the studies, suggesting that recombination is an important driving force for genetic evolution of bovine noroviruses, as already evidenced in human strains.

Genetic comparisons between entire genotype 2 sequences detected on a thirty-years period allowed to highlight the genomic regions the more subjected to genetic evolution. Surprisingly for such RNA viruses, very low genetic divergence was observed. It was confirmed when the substitution rates/site/year were inferred by Bayesian methods on both the entire genome of genotype 2 bovine noroviruses or their single capsid protein coding gene. Interestingly, they notably showed that bovine noroviruses seemed to evolve slower than some human ones, allowing the development of a concept of different evolution driving forces depending on the genogroup/genotype in the genus *Norovirus*.

Altogether, the results helped the Public Health authorities to presently refute or attenuate the zoonotic risk associated to bovine noroviruses, confirmed bovine noroviruses as important pathogens for bovine health and provided fundamental data on the genetic evolution for bovine noroviruses.

26.

DNA methylation: A positive regulator of viral telomerase RNA expression in Marek's Disease Virus?

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Marek's disease virus (MDV) is an *alphaherpesvirus* of chickens that induces T lymphomas and tumors within four weeks of infection. Because of its high replication rate and its significant mortality, this avian virus represents an important warning to the poultry industry. Moreover, MDV is used as a unique model for studying tumor T cells lymphomas development.
MDV life cycle, like those of other *herpesviruses*, is composed of three alternative stages: lytic infection, latency and reactivation. During the lytic phase and the reactivation, the virus replicates actively in target cells, whereas in the latency phase, it persists in the host cells without replicating. During this last phase, MDV expresses a limited subset of transcripts. All of them are involved in the maintenance of the latent state and/or in the virus induced-tumorigenesis. One of the latent virus-encoded oncogenes involved in the transformation process is the viral telomerase RNA subunit (vTR). By interacting with the cellular telomerase reverse transcriptase (TERT), vTR reconstitutes telomerase activity and maintains the length of telomeres. In this study, we investigated the role of DNA methylation in the regulation of vTR expression during the different stages of MDV infection.

The promoter of the vTR gene was first analyzed to determine its precise DNA methylation pattern through Bisulfite Genomic Sequencing Analysis at relevant *in vitro* and *in vivo* conditions of MDV infection. We observed that the vTR promoter presents discrimative patterns between the different stages of the infection. CpG methylation level was indeed high (80%) in latency while it was lower (18%) during reactivation and was at 0% in lytic infection. *In vivo* studies on peripheral blood leucocytes infected with MDV revealed that vTR promoter displayed a DNA methylation percentage close to 0%.
The functional impact of DNA methylation was then assessed on telomerase activity and vTR expression in latently infected cells. Interestingly, we showed that the 5'azacytidin induced demethylation of the viral genome negatively influences the activity of the telomerase. In order to confirm this result, we also investigated the effect of CpG methylation on vTR promoter activity by luciferase assay. Moreover, we showed that this transcriptional activation associated with a methylated promoter was specific of the vTR gene. All the others lytic and latent genes analysed (pp38, ICP27 and miR9/4 transcript) presented indeed a strong negative correlation between their DNA methylation pattern and their promoter activity.
Taken together, these results suggest that DNA methylation positively influences the expression level of vTR.

27.
Viral semaphorin encoded by *Alcelaphine herpesvirus 1* is a secreted glycoprotein that is not essential for malignant catarrhal fever induction

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Alcelaphine herpesvirus 1 (AlHV-1) is a gammaherpesvirus that persists asymptomatically in wildebeest. However, AlHV-1 transmission to a large number of ruminants, including cattle and the experimental rabbit model, results in the development of a lethal lymphoproliferative disease named malignant catarrhal fever (MCF). The A3 gene of AlHV-1 encodes a putative semaphorin-homolog protein termed AlHV-Sema. The family of semaphorins includes glycoproteins that are characterized by a conserved amino-terminal Sema domain and have been shown to act on cytoskeleton *via* plexins or integrins as receptors. First identified as axonal guidance factor, they have also been involved in the immune system. The cellular homolog semaphorin 7A (Sema7A) can notably stimulate macrophage pro-inflammatory cytokine secretion and is involved in T cell-mediated inflammatory responses. In addition, viral semaphorin A39R of the poxvirus Vaccinia has been shown to inhibit cross-presentation of antigens by dendritic cells to $CD8^+$ T cells through action on cytoskeleton and inhibition of phagocytosis. In this study, we sought to investigate the role of AlHV-sema during MCF and during viral infection. First, we used Fc-tagged AlHV-Sema constructs and showed that AlHV-Sema is a 115 kDa secreted glycoprotein during viral infection *in vitro*. Next, we addressed the role of AlHV-Sema during viral infection. We produced A3-deleted and A3-non-sense viral strains and their respective revertant control strains. Absence of A3 expression did not significantly affect virus growth *in vitro*. These viruses were further used to infect rabbits using intranasal inoculation. A3-deleted or A3 non-sense viruses induced MCF in rabbits similarly to WT and revertant viruses, indicating that A3 is not essential for the development of MCF. To further investigate whether AlHV-Sema shares similar functions with the vertebrate homolog Sema7A, we used the Fc-tagged AlHV-Sema recombinant protein in binding assays on different cell lines. Though binding could not be detected on murine macrophages, we detected specific binding of AlHV-Sema to the surface of several bovine cell lines, including fibroblasts, macrophages and monocytes. AlHV-Sema binding induced cytoskeleton condensation with reduced F-actin cables. Further analyses are currently in progress to determine the implication of AlHV-Sema binding on these cells.

28.

An Essential Role for γ-herpesvirus Latency-associated Nuclear Antigen Homolog in an Acute Lymphoproliferative Disease of Cattle

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Wildebeests carry asymptomatically *alcelaphine herpesvirus 1* (AlHV-1), a γ-herpesvirus inducing malignant catarrhal fever (MCF) to several ruminant species (including cattle). This acute and lethal lymphoproliferative disease occurs after a prolonged asymptomatic incubation period following transmission. Our recent findings with the rabbit model indicated that AlHV-1 infection is not productive during MCF. Here, we investigated whether latency establishment could explain this apparent absence of productive infection and sought to determine its role in MCF pathogenesis. First, whole-genome cellular and viral gene expression analyses were performed in lymph nodes of MCF-developing calves. Whereas a severe disruption in cellular genes was observed, only 10% of the entire AlHV-1 genome was expressed contrasting with the 45% observed during productive infection *in vitro*. *In vivo*, the expressed viral genes included the latency-associated nuclear antigen homolog ORF73 but none of the regions known to be essential for productive infection. Next, genomic conformation analyses revealed that AlHV-1 was essentially episomal, further suggesting that MCF might be the consequence of a latent infection rather than abortive lytic infection. This was further supported by the high frequencies of infected $CD8^+$ T cells during MCF using immunodetection of ORF73 protein and single-cell RT-PCR approaches. Finally, the role of latency-associated ORF73 was addressed. A lack of ORF73 did not impair initial virus replication *in vivo*, but rendered AlHV-1 unable to induce MCF and persist *in vivo* and conferred protection against a lethal challenge with a wild-type virus. Together, these findings suggest that a latent infection is essential for MCF induction.

29.

Study of the epigenetic control of chicken miR-126, a tumor suppressor microRNA downregulated in cells transformed and latently infected with Marek's disease virus.

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Marek's disease is an avian model of herpesvirus induced lymphoma. The causative agent, the Marek's disease virus (MDV) is an alphaherpesvirus that establishes a latent infection in T CD4+ lymphocytes. Previous studies showed that MDV latent infection produces a panel of functional viral microRNAs (miRNAs) involved either in the virus induced oncogenesis or in the regulation of the viral cycle. In addition, these studies indicated that besides encoding viral miRNAs, the virus infection affected the expression of several cellular miRNAs with some of the chicken miRNAs up-regulated (miR-21, miR-221 and miR-222) while other were downregulated (miR-155, miR-223 and miR-126). In this present study, we investigated the miR-126 downregulation and asked whether epigenetic modifications were involved in this repressed expression. The human homolog of miR-126 has been shown to be repressed in several human cancers and has been identified as a potential tumor suppressor. The specific aims are to map and to characterize epigenetic signatures found in the promoter of chicken host gene containing the precursor of miR-126 (premiR-126). This gene encodes a proangiogenic protein, *EGFL7* (endothelial growth factor-like 7) and contains the premiR-126 sequence into an intronic sequence.

To study the DNA methylation pattern of miR-126 and its host gene, *in silico* analysis was used to map CpG islands (regions that mainly undergo DNA methylation) in miR-126 genomic context. Two CpG islands were found. Bisulfite Genomic Sequencing Assays targeting the two islands were set up and used to determine the methylation level in different chicken organs and in MDV transformed lymphocytes. Sequencing data indicated that MDV transformed cells possessed the highest level of methylation with 95 % of methylated CpG sites. In comparison, cells isolated from different organs (brain, testis, lungs, heart and spleen) collected in three mock infected chickens showed respectively 84, 82, 70, 56 and 55 % of methylated CpG sites. The next step is in progress and aims at establishing a correlation between the observed CpG methylation pattern and the expression level of miR-126 in the corresponding samples. In regard with the high level of methylation found in the miR-126 gene environment, MDV transformed cells were treated with 5'-Azacytidine, a DNA methyltransferase inhibitor. The treatment inhibited the proliferation of MDV transformed cell line. The analysis on miR-126 expression induced by this treatment is in progress. To precisely map the potential promoter controlling miR-126 and *EGFL7* expression, we explored the transcriptional start sites (TSS) by using 5' RACE-PCR (5' Rapid Amplification of cDNA Ends). Analysis of sequences is in progress. The comparison of TSS mapping in different contexts (high versus low methylation pattern) will reveal whether DNA methylation induces or not alternative initiation of transcription.

Preliminary conclusion indicates that Marek's disease virus induces a DNA hypermethylation of miR-126 host gene. Further experiment will determine whether this epigenetic signature is responsible of the miR-126 repression.

30.

Is there a link between the multiple activities of Theiler's virus Leader (L) protein?

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Theiler's murine encephalomyelitis virus (TMEV) is a picornavirus which produces a very short protein that is cleaved from the N-terminal end of the viral polyprotein. This non-structural protein is called L (for *Leader*). In spite of its very small size (76 amino acids), 4 "domains" have been recognized in this protein: a N-terminal zinc finger, an acidic domain, a serine/threonine-rich domain and a C-terminal domain that is called Theilodomain since it is conserved in Theilovirus L proteins but absent from the related L protein of EMCV. L of TMEV and EMCV are multifunctional proteins that interfere with numerous cell functions: interferon (IFN) and chemokine production, apoptosis induction, stress granules formation, protein synthesis, MAP kinase activation and nucleo-cytoplasmic trafficking of cellular proteins and RNA.

This work aimed at defining whether the various activities of the L protein are coupled (i.e. if they all derive from a single master activity of L). Therefore, we constructed a series of mutants where single amino acids were converted into Alanines. In addition, we mutated highly conserved amino acids D30 and W33 that were shown, in the case of EMCV, to interact with RAN GTPase, a critical player of nucleo-cytoplasmic trafficking (Bacot-Davis and Palmenberg, 2013).

As a first screen, we introduced these mutations in a bicistronic vector that enables co-expression of L and GFP. Since the wild-type L (L^{wt}) inhibits protein synthesis, it quickly shuts off the expression of GFP from the bicistronic vector. To test the influence of the L mutations on the ability of the protein to shut-off the expression of GFP, we transfected Neuro 2A cells with the various bicistronic constructs and tested GFP expression by flow cytometry. This analysis confirmed the major role of the Theilodomain and, to a lesser extent, of the zinc finger domain to protein synthesis shut-off. Interestingly, conserved residues that were reported to interact with RAN GTPase (D30 and W33) did not contribute to host cell shut-off, suggesting that the L protein has distinct functions. Surprisingly other well-conserved residues (F23, L27 and P35) did not affect host protein shut-off, suggesting that these residues might play an additional role. Selected mutations were introduced in an infectious virus clone, to study the impact of these mutations on other L functions (inhibition of IFN transcription, nucleo-cytoplasmic trafficking and stress granule formation). This analysis is in progress.

In conclusion, our data suggest that the multiples activities of the leader protein are not linked. It is surprising, however, that such a small protein can play as many functions.

31.

Mechanisms of Entry of the Human T-Lymphotropic Virus type I and Bovine Leukemina Virus into its Host Cells

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HumanT-lymphotropic virus type 1 (HTLV-1) and bovine leukemia virus (BLV) are two related δ-retroviruses sharing a similar genomic organization and infecting cells from the hematopoietic system. Although significant progress has been made in the mechanisms of cell transformation, little information is available on the early steps of viral infection. In particular, the post-entry steps consecutive to receptor binding such as internalization, intracellular trafficking and preintegration complex formation are largely unknown. In this study, we propose to characterize the early steps of infection by HTLV-1 and BLV. We study the different pathways that internalize molecules from the surface of eukaryotic cells (clathrin-mediated endocytosis, caveolin-mediated endocytosis and pathways independent of clathrin and caveolin). To address these questions, we compare the two modes of viral infection: cell-to-cell infection and cell-free infection. The internalization pathways will be evaluated by the internalized virions in presence of pharmacological inhibitors or RNA silencing and dominant-negative proteins of clathrin and caveolae dependent and independent endocytosis. Further, the mechanisms of internalization will be characterized by colocalization, co-immunoprecipitation and electron microscopy studies.

Together, these experiments will provide useful information about the modes of early infection by δ-retroviruses.

32.

The IL-10 homologue encoded by cyprinid herpesvirus 3 is essential neither for viral replication *in vitro* nor for virulence *in vivo*

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Cyprinid herpesvirus 3 (CyHV-3), a member of the family *Alloherpesviridae,* is the causative agent of a lethal disease in common and koi carp. CyHV-3 ORF134 encodes an interleukin-10 (IL-10) homologue. The present study was devoted to this ORF. Transcriptomic analyses revealed that ORF134 is expressed as a spliced gene belonging to the early-late class. Proteomic analyses of CyHV-3 infected cell supernatant demonstrated that the ORF134 expression product is one of the most abundant proteins of the CyHV-3 secretome. To investigate the role of ORF134 in viral replication *in vitro* and in virulence *in vivo*, a deleted strain and a derived revertant strain were produced using BAC cloning technologies. The recombinant ORF134 deleted strain replicated in *vitro* comparably to the parental and the revertant strains. Infection of fish by immersion in water containing the virus induced comparable CyHV-3 disease for the three virus genotypes tested (wild type, deleted and revertant). Quantification of viral DNA by real time TaqMan PCR (in the gills and the kidney) and analysis of carp cytokine expression (in the spleen) by RT-qPCR at different times post-infection did not revealed any significant difference between the groups of fish infected with the three virus genotypes. Similarly, histological examination of the gills and the kidney of infected fish revealed no significant differences between fish infected with ORF134 deleted virus versus fish infected with the control parental or revertant strains. All together, the results of the present study demonstrate that the IL-10 homologue encoded by CyHV-3 is essential neither for viral replication *in vitro* nor for virulence in common carp.

33.

Interference of Pseudorabies virus with Erk 1/2 signaling in T lymphocytes

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The subfamily of Alphaherpesviruses is the largest subfamily of Herpesviruses. Human alphaherpesviruses include the Herpes Simplex Virus (HSV) and Varicella Zoster Virus (VZV). The porcine Pseudorabies Virus (PRV) is closely related to HSV and VZV, and PRV is commonly used as a study model to investigate conserved aspects of alphaherpesvirus biology (Pomeranz et al., 2005, J Virol).

Alphaherpesviruses have been shown to interfere with signaling in T lymphocytes (Sloan et al., 2006, J Immunol). The mechanisms used by alphaherpesviruses to modulate T-cell responses remain largely unknown. Pseudorabies virus (PRV) glycoprotein E (gE) is an important mediator of virulence and viral spread. We previously showed that gE behaves to some extent like an immune receptor. Indeed, like immune receptors, it contains an ITAM (immune receptor tyrosine activation motif)-like domain that can be phosphorylated upon antibody crosslinking (Desplanques et al., 2007, Virology). Hence, gE expression may potentially result in aberrant signaling in infected leukocytes.

The aim of the current study was to evaluate whether gE indeed affects signaling in T cells, focusing on the MAP kinase ERK 1/2. Jurkat T cells inoculated with PRV wild type (WT) showed an increase in ERK 1/2 phosphorylation when compared to mock-infected cells. To investigate the involvement of gE in this process, two mutant viruses were used: PRV 91 (ΔgE) (Card et al., 1992, J Virol) and PRV 107 (gEΔcytoplasmic domain)(Tirabassi and Enquist, 1998, J Virol). PRV 91 (lacks gE) was unable to induce ERK 1/2 phosphorylation, indicating that gE is required for ERK 1/2 phosphorylation. Unexpectedly, infection with PRV 107 (lacking the cytoplasmic domain of gE) resulted in ERK 1/2 phosphorylation equivalent to WT PRV infection. These results show that during PRV infection of Jurkat T cells, gE is required to induce ERK 1/2 phosphorylation but not through its cytoplasmic domain.

In further support that the extracellular domain of PRV gE is responsible for triggering ERK 1/2 phosphorylation in Jurkat T cells, addition of recombinant gE protein (10 μg/ml per 10^6) to Jurkat T cells was sufficient to induce ERK 1/2 phosphorylation within 2 min post addition. Similarly, stimulation of porcine primary T lymphocytes with recombinant gE protein (100 μg/ml per 10^7) showed an increase in ERK 1/2 activation at 5 min post stimulation, corroborating the results observed in Jurkat T-cells.

We observed that gE-mediated ERK 1/2 signaling affects T cell-mediated production of the anti-inflammatory cytokine IL10. Infection with PRV 91 (lacks gE) led to a significant decrease in IL10 levels in relation to PRV WT infection.

In conclusion, PRV induces ERK 1/2 phosphorylation through the extracellular domain of its gE protein, which appears to affect IL10 production. These results may point to a new immune evasion strategy of the virus. Currently, we are further investigating the molecular mechanism of gE-induced ERK 1/2 phosphorylation.

34.

Schmallenberg virus infection of pigs

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Abstract

Schmallenberg virus (SBV) is a newly emerged virus responsible for a non-specific syndrome in adult cattle including high fever, decrease in milk production and severe diarrhoea. It also causes reproductive problems in cattle, sheep and goat including abortions, stillbirths and malformations. The role of pigs in the epidemiology of SBV has not yet been evaluated while this could be interesting seen their suggested role in the epidemiology of the closely related Akabane virus. To address this issue, four 12 week old seronegative piglets were subcutaneously infected with 1ml of SBV infectious serum (FLI) and kept into contact with four non-infected piglets to examine direct virus transmission. Throughout the experiment blood, swabs and feces samples were collected and upon euthanasia at 28 dpi different organs (cerebrum, cerebellum, brain stem, lung, liver, iliac lymph nodes, kidney and spleen) were

sampled. No clinical impact was observed and all collected samples tested negative for SBV in rRT-PCR. Despite the absence of viremia and virus transmission, 2 out of 4 infected piglets seroconverted but only low amounts of neutralizing antibodies were found. The limited impact of SBV infection in pigs was further supported by the absence of neutralizing anti-SBV antibodies in field collected sera from domestic pigs and indications of only a low seroprevalence (4%) in wild boar were found.

In conclusion, SBV infection of pigs can induce seroconversion but is ineffective in terms of virus replication and transmission indicating that pigs have no obvious role in the SBV epidemiology.

35.

The mechanism of behavioral fever induced by Cyprinid herpesvirus 3 in common carp (*Cyprinus carpio* L.).

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Fever in response to infectious or inflammatory agents is an ancestral mechanism of innate immunity existing in all vertebrates including fish. Since fish cannot rely on endogenous thermoregulatory mechanisms to control their body temperature, they mainly use behavioural thermoregulation stimulating the individual to reside in a warmer environment, a phenomenon called "behavioural fever". Cyprinid herpesvirus 3 (CyHV-3) is the causative agent of a lethal disease in common and koi carp. Interestingly, the outcome of CyHV-3 infection is highly dependent on the temperature of the water in which carp are maintained. Outbreaks of CyHV-3 occur only when water temperature is between 18°C and 28°C. This study aimed to determine whether carp are able to express behavioural fever as a consequence of CyHV-3 infection and what are potential mechanisms of this process.

To study the mechanism of behavioural fever in carp during CyHV-3 infection we developed multi-chamber aquaria with a temperature gradient (24°C-28°C-34°C) and informatics system to monitor the position of the fish. The results of our study demonstrated that: **1) CyHV-3 infection induced behavioral fever in carp.** Infected fish migrated to the compartment with the highest temperature (34°C) and stayed there until control of the disease. **2) Behavioural fever induced by CyHV-3 is salutary.** Migration of infected fish to 34°C resulted in the absence of mortality while a mortality of 100% and 80% was observed when the fish were maintained in control tanks at 24°C and 28°C, respectively. **3) PGE2 plays a role in development of behavioural fever.** Inhibition of PGE2 production by COX1/2 inhibitor (Indomethacin) impaired the expression of behavioural fever. **4) One of the viral genes modulates behavioural fever.** A series of experiment performed with a recombinant strain deleted for ORF134 (encoding a viral IL-10 homologue) demonstrated that the deleted strain induced behavioural fever earlier than the control revertant strain. This resulted in a faster control of viral replication by fish infected with the deleted recombinant compared to the revertant strain. Interestingly, this experiment demonstrated that the ORF134 gene confers an advantage to the virus when tested in gradient tank while previous studies demonstrated no effect of this gene when infection were performed at 24°C. **5) TNF-α plays an essential role in the expression of behavioural fever induced by CyHV-3 infection.** Transcriptomic analysis of the expression of cytokine genes (IL-1β, IL-6, IFN-γ, and TNF-α) during the course of CyHV-3 infection demonstrated that the expression of behavioural fever correlated with the up-regulation of TNF-α gene, while other

cytokine genes were already upregulated earlier. Interestingly, injection of CyHV-3 infected fish with anti-carp TNF-α antibodies blocked migration of infected fish to 34°C, thereby demonstrating the essential role of TNF-α in the behavioural fever induced by CyHV-3 infection.

36.

Immunological tools for the measurement of the immune response against respiratory viral infections of poultry (mainly AI & ND)

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Our laboratory has developed immunological tools to measure the immune response against respiratory viral infection in poultry, mainly Avian Influenza (AI) and Newcastle Disease (ND). Purified ND and AI recall antigens were produced after whole virus concentration, chemical inactivation and viral proteins dissociation. These were used for the evaluation of the specific cell-mediated immunity (CMI) in the chickens after *ex vivo* activation of lymphocytes from the spleen and the peripheral blood and measurement of ChIFNγ production by ELISA-ChIFNγ test previously developed in our lab. Following the improvement of lymphocyte isolations from the duodenum, the lung and the trachea, we are now able to measure the cellular immune response induced in the digestive and the respiratory tract after NDV or AIV vaccination or infection. Technologies such as FACS, ELISPOT and real-time RT-PCR are available or under development for the detection of cytokines and characterization of immune cells after ND and AI vaccination or infection. Additionally, NDV- and AIV-specific IgG, IgM and IgA ELISA was developed to evaluate the antibody-mediated immunity in the digestive and the respiratory tract after lung washings and *ex vivo* tissue culture of duodenum and trachea.

37.

Identification and characterization of new Sp1 sites located in the R region of the Human T-lymphotropic Virus 1 (HTLV-1) Long Terminal Repeat

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HTLV-1 infection is characterized by viral latency in the large majority of infected cells and by the absence of viremia. These features are thought to be due to the transcriptional repression of viral expression in vivo. Specific protein 1 (Sp1) binds to more than 1000 different cellular promoters and regulates the expression of numerous genes involved in cell proliferation, apoptosis, and differentiation. In silico analysis of the nucleotide sequence of the HTLV-1 LTR revealed the presence of two new potential Sp1 binding sites within the R region. We demonstrated that the Sp1 and Sp3 transcription factors bound in vitro to these sites by EMSAs and supershift experiments. We also performed competition assays with a probe corresponding to a Sp1 binding site consensus in order to compare Sp1 binding affinity for the four previously reported and the two newly identified Sp1-binding sites located in the HTLV-1 promoter. Point mutations of the known and newly identified Sp1 sites were introduced in the HTLV-I LTR cloned in either the sense or the antisense orientation in the context of an episomal reporter vector. We demonstrated that the Sp1 sites interfered with both the sense transcription from the 5?LTR and the antisense transcription from the 3?LTR (necessary for HBZ expression in vivo). ChIP experiments to evaluate in vivo Sp1 binding to the HTLV-I LTR U3 region are currently under investigation. Our results demonstrate the presence of two new functional Sp1 binding sites located in the R region of the HTLV-I LTR.

38.

SENSITIVITY AND PERMISSIVITY OF CYPRINUS CARPIO TO CYPRINID HERPESVIRUS 3 INFECTION ACCORDING TO HOST ONTOGENESIS.

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Cyprinid herpesvirus 3 (CyHV-3) is the causative agent of a lethal disease in common carp (*Cyprinus carpio carpio*) and koi (*Cyprinus carpio koi*). In the present study, we investigated the sensitivity and the permissivity of common carp to CyHV-3 infection according to host ontogenesis (from hatching to juvenile stage). Each developmental stage was infected by immersion in water containing the CyHV-3 recombinant LUC strain expressing firefly luciferase as a reporter gene. In vivo imaging system (IVIS) analyses performed 24 h and 72 h post-infection demonstrated that carp is sensitive and permissive to CyHV-3 infection from hatching onwards; however, both sensitivity and permissivity increase with ontogenesis. IVIS data were confirmed by observation of the mortality induced by CyHV-3 following infection at the different developmental stages. Finally, to further compare the pathogenesis of CyHV-3 infection according to host development, we produced a CyHV-3 recombinant strain expressing beta-galactosidase as a reporter gene. This recombinant allowed us to detect the viral infection both at macroscopic and microscopic levels. Its use revealed that independently of the host stage development, the skin covering the body and the fins is the major portal of entry after fish immersion in water containing the virus.

40.

Abstract

High-throughput analysis of human cytomegalovirus genetic diversity reveals variation in coding capacity and extensive recombination

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Human cytomegalovirus (HCMV) is a ubiquitous herpesvirus that causes serious sequelae in immunocompromised patients and newborns. The coding capacity and diversity of its 235 kbp genome is still not fully comprehended and there is a pressing need to characterize genomic contents in clinically representative strains. We therefore have developed a procedure for the high-throughput generation of full genome sequences from minimally passaged clinical isolates. This procedure relies on low number passaging of clinical strains on human fibroblasts, followed by digestion of cellular DNA and purification of viral DNA. After multiple displacement amplification, highly pure viral DNA was generated. Validation experiments have shown that the consensus sequences derived from these extracts using different next-generation sequencing platforms are representative for the virus population present in the original clinical material. Full genome analysis of 100 clinical isolates has revealed extensive genetic variability in several regions of the HCMV genome. In addition, gene-disrupting mutations were identified in a set of 21 genes, including the viral interleukin-10 encoding gene UL111A. Consequently, HCMV coding capacity seems to be variable among clinical isolates and this variation could have clinical significance. Finally, phylogenetic analysis highlighted the predominant role of recombination in the evolution of HCMV genetic diversity, which provides the virus with a very powerful mechanism of adapting to its changing environment through the exchange of alleles.

42.

HEPATITIS E VIRUS INFECTION IN WILD BOARS AND HUMANS IN BELGIUM

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Hepatitis E virus (HEV) possesses four genotypes. In Europe, genotype (G) 3 mainly circulates and its route of transmission is highly suspected to be zoonotic. The aims of this study were to obtain data on apparent viroprevalence and seroprevalence in wild boar and to compare the different strains identified in wild boar and human in Belgium.

For the detection of the viral infection, a nested RT-PCR, an ELISA and a Western blot were used. A sample of 383 wild boar sera and 69 sera and 61 livers from young wild boars was obtained during the hunting season in 2010. The human samples were obtained by the Belgium Scientific Institute of Public Health and concerned all the sera samples sent by physicians for HEV diagnosis in Belgium.

An apparent seroprevalence of 33% (±4.6; 125/383) was obtained in wild boars. Five out of 61 livers and 4/69 sera of young wild boars were detected viropositive. The sequences obtained belonged to G3f and 3c. In humans, 25/340 sera in 2010 and 32/437 in 2011 were IgM positive and, from these 25 and 32 sera, 10 and 24, respectively, were viropositive. From these, 4 belonged to G1, 7 to G3 and 1 to G4.

In conclusion, the high HEV seroprevalence in wild boars in Belgium raises zoonotic concern about HEV transmission. The HEV sequences obtained from wild boars were for most of G3f like most of the human HEV sequences. Therefore, these data are in agreement with the situation observed in other European countries and the links between HEV infection in pigs, wild boars and humans need to be further analysed to support the hypothesis of a zoonotic transmission in Belgium.

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43.

An Enterovirus 71 mouse model with central nervous system involvement

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To aid the development of novel antivirals against enterovirus 71 (EV71), a new *in vivo* model was desired. To this end, an EV71 clinical isolate was passaged in the brains of mouse pups to adapt it to the new host. Subsequent infection of Severe Combined Immune Deficiency syndrome (SCID) mice with the mouse adapted as well as the parent EV71 strain resulted in paralysis, coordination and balance problems, although the mouse adapted variant caused disease faster than the non-mouse adapted virus. Using qPCR, it was determined that the mouse adapted virus was present in high levels in the brain and spinal cord whereas in other tissues only low levels were detected. Upon further investigation, using immunohistochemistry, it was demonstrated that in the CNS specifically the motor neurons were positive for EV71 whereas surrounding cells were not infected. Preliminary sequencing results showed the presence of a mutation in the gene of viral protein VP2 which resulted in an amino acid substitution at position 135. The importance of this mutation in EV71 induced pathology is being investigated.

44.

HIV-1 Vpr N-terminus is critical for efficient Vpr de novo expression and infectivity in non-activated CD4+ T cells

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The HIV-1 Vpr protein is a 14 kDa accessory protein, required for efficient replication in macrophages. Vpr is incorporated into HIV virions, believed to participate in the docking of the HIV-1 pre-integration complex to the nucleus and to facilitate it's transport through the nuclear pore. By inducing G2 arrest, Vpr favors transcription from the HIV-1 LTR, which it also transactivates.

We noticed two N-terminal amino acids of the HIV-1, SIVmac and SIVcpz Vpr proteins are fully conserved. This N-terminal motif is predicted to be a NatB substrate motif expected to lead to full Nt-acetylation of the protein, but it also allows the conservation of the Kozak consensus sequence critical for efficient protein translation. Nt-acetylation, one of the most common protein modifications in eukaryotes, is believed to affect protein stability, degradation and function. We set out to determine whether Vpr's amino-terminal sequence is conserved for functional Nt-acetylation, and/or to allow optimal protein translation.

Nt-acetylation of Vpr was detected by COFRADIC (COmbined FRActional Diagonal Chromatography) in HIV-1 infected Jurkat cells, and confirmed in transfected cells. Point mutations were introduced in the Vpr protein, abolishing Nt-acetylation in part or completely, and modifying the Kozak consensus sequence. Mutants were cloned into a retroviral vector. Protein expression, translation, degradation, localization, and function (G2 arrest, apoptosis and transactivation) was evaluated in Jurkat and 293T cells. In parallel, Vpr protein was mutated similarly in HIV-1. Vpr WT and mutant protein expression, incorporation into viral particles and viral infectivity was evaluated in non-activated and activated peripheral blood CD4+ T cells.

Results show Vpr protein expression levels to be reduced by mutations affecting Nt- acetylation. However, when protein translation was inhibited, mutants versus wild-type Vpr were not degraded faster, suggesting Nt-acetylation does not protect the protein from degradation. The reduced steady state levels could be the result of mutated Kozak consensus sequence on translation efficiency, still under investigation. Mutants retained Vpr functions tested, also suggesting Nt-acetylation is not necessary for Vpr function per se. However, in the context of replication competent HIV-1 virus, the reduced mutant Vpr levels in infected cells resulted in diminished incorporation into HIV particles. In non-activated T cells, infection rates with Vpr N-terminus mutants were reduced down to levels of Vpr deleted HIV-1.

Altogether, our results show N-terminus conservation is important for sufficient Vpr protein levels in infected cells/virions and efficient infection of non-activated CD4+ lymphocytes is dose-dependent on Vpr expression levels. Nt-acetylation does not affect protein function per se, nor does it influence protein half-life. Our work demonstrates the importance of Vpr for infection of non-activated primary CD4+ T cells.

45.

Porcine, murine and human sialoadhesin (Sn/Siglec-1/CD169): portals for porcine reproductive and respiratory syndrome virus entry into target cells

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Porcine sialoadhesin (pSn; a sialic acid-binding lectin) and porcine CD163 (pCD163) are molecules that facilitate infectious entry of porcine reproductive and respiratory syndrome virus (PRRSV) into alveolar macrophages. In this study, it was shown that murine Sn (mSn) and human Sn (hSn), like pSn, can promote PRRSV infection of pCD163-expressing cells. Intact sialic acid-binding domains are crucial, since non-sialic acid-binding mutants of pSn, mSn and hSn did not promote infection. Endodomain-deletion mutants of pSn, mSn and hSn promoted PRRSV infection less efficiently, but also showed markedly reduced expression levels, making further research into the potential role of the Sn endodomain in PRRSV receptor activity necessary. These data further complement our knowledge on Sn as an important PRRSV receptor, and suggest – in combination with other published data – that species differences in the main PRRSV entry mediators Sn and CD163 do not account

46.

THE ORF25 GENE FAMILY OF CYPRINID HERPESVIRUS 3 ENCODES NON ESSENTIAL STRUCTURAL GLYCOPROTEINS

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Cyprinid herpesvirus 3 (CyHV-3) is the aetiological agent of a lethal disease in common and koi carp. Since its emergence, in the late 1990s, CyHV-3 caused severe economic losses in carp culture industries worldwide. In addition to its economic importance, CyHV-3 has several qualities as a fundamental model of infection. Sequencing of CyHV-3 genome revealed that it encodes a family of type I membrane proteins called ORF25 family. This family is composed of 6 paralogous sequences: ORF25, ORF65, ORF148 and ORF149 encode structural proteins that are non-essential for viral replication *in vitro*, while ORF26 and ORF27 were described as pseudogenes. Recent sequencing of field strains revealed that they encode, in contrast to laboratory strains, an ORF27 sequence compatible with the expression of a type I membrane protein. This observation raised the hypothesis that CyHV-3 propagation *in vitro* could lead to selection of CyHV-3 mutants with an invalidated ORF27 as a consequence of a negative effect of full length ORF27 expression product on viral growth *in vitro*. The goal of the present project was to test this hypothesis. To reach this goal, ORF27 recombinant strains encoding a full length ORF27 were derived from the laboratory FL BAC strain (encoding a truncated ORF27) using BAC cloning and prokaryotic recombination technologies. The recombinants produced were analysed at the genome and proteomic levels. Genome analyses demonstrated that they had the correct molecular structure; while proteomic analyses demonstrated that the recombinant strains encoding a full length ORF27 (in contrast to the parental FL BAC strain) express a related protein that was detected in semi-purified virions. To investigate whether the expression of a full length ORF27 could have a negative effect on viral growth *in vitro*, the recombinant strains produced (encoding a full length ORF27) were compared to the parental strain (encoding a truncated form of ORF27) through multistep

growth curves and plaque size assay. None of the assays revealed a significant effect of ORF27 mutation on *in vitro* growth. The present study demonstrates that the ORF27 paralogue of the ORF25 family is a gene and not a pseudogene as described earlier. It increases the composition of the ORF25 family to 5 paralogous transmembrane type I proteins further increasing the scientific interest of this unique viral family.

47.

Comparison of Ion Torrent PGM and Illumina MiSeq next generation sequencing for influenza A virus quasispecies analysis

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The RNA genome of influenza viruses has a relatively high mutation rate because the viral RNA-dependent RNA polymerase lacks intrinsic 3'-5'-exonuclease activity, and therefore proof-reading function. As a result, the virus presumably exists as a large group of closely related genotypes, also called quasispecies. To determine the composition of such a viral quasispecies, a very accurate and sensitive sequencing technique is needed. Next generation sequencing technology now allows such analysis.

Here we compared the suitability of two benchtop sequencers for influenza quasispecies sequencing: the Illumina MiSeq (2*250 bp) sequencing-by-synthesis technique and the Ion Torrent PGM (400 bp) semiconductor sequencing technique.

We first validated the accuracy and the sensitivity of both sequencing techniques using plasmid DNA, i.e. a much more stable genetic information carrier compared with the viral RNA genome. As reference, a wild type and a mutant plasmid containing two introduced silent mutations were used. We deduced the accuracy of the two sequencing platforms by sequencing both plasmids in duplicate on both instruments. Globally, the Ion Torrent PGM generated more sequencing errors, with the majority being insertions and deletions, mostly in homopolymer regions. In contrast, the Illumina MiSeq sequencing reads have in general a slightly higher number of substitution errors. The sensitivity was determined by mixing the wild type and mutant plasmid in different ratios and we subsequently verified if these ratios were reflected in the sequencing reads. For the Illumina MiSeq, the sensitivity has been determined as 0.5%. The same cut-off can only be used for Ion Torrent PGM when we excluded indel errors. Due to its high sensitivity, next generation sequencing is the preferred technique to study the influenza quasispecies.

In addition, the suitability of both benchtop sequencers for determining the quasispecies composition of influenza A PR8 virus that had been grown *in vitro* on MDCK cells was evaluated. For this, we reverse transcribed the influenza RNA genome using an optimized RT-PCR protocol, leading to equal amplification of all eight genomic segments. Both sequencing techniques obtained uniform coverage across each and between all eight genome segments. The most variation in the virus quasispecies present in the virus stock was detected by both techniques in the HA segment. Surprisingly, both techniques detected also some non- synonymous variation in the conserved PB1 and NP segment. In accordance with the plasmid-derived data, more sequencing errors (majority insertions and deletions) were generated by the Ion Torrent PGM than with the Illumina MiSeq. We also conclude that the Illumina MiSeq platform has a slightly higher accuracy than the PGM technology to study the RNA virus sequence diversity.

48.

Dual role of the cellular cofactor CTIP2 in HIV-1 latency

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Our previous studies reported that the cellular cofactor CTIP2 (Bcl11b) favors the establishment of HIV-1 post-integration latency in microglial cells, the main reservoirs of virus in the brain. CTIP2 recruits chromatin-modifying enzymes to promote a heterochromatin environment at the HIV-1 transcriptional promoter. This epigenetic control clearly favors establishment of latency, but cannot explain the persistence of the reservoirs, which depends on cellular counteractions aimed at preventing the environmental stimuli. Interestingly, these events rely on the positive transcription elongation factor b (P-TEFb) (CyclinT1/Cdk9) function.

Our objective was to evaluate the involvement of CTIP2 in mechanisms that prevent reactivation of HIV-1 latent proviruses. More specifically, we studied the effect of CTIP2 on P-TEFb function. To this end, we used complementary biological, biochemical, molecular and genome wide approaches.

Purification of the P-TEFb-associated complexes and co-immunoprecipitation experiments demonstrated that CTIP2 interacts with an inactive P-TEFb complex containing HEXIM1 and the 7SK snRNA. CTIP2 associates directly with HEXIM1 and, via the loop 2 of the 7SK snRNA, with P-TEFb. Kinase assays demonstrated that CTIP2 inhibits the CDK9 kinase activity of P-TEFb *in vitro* and *in vivo*. In addition, chromatin immunoprecipitation experiments showed that CTIP2 recruits the inactive P-TEFb complex to the HIV-1- but also to cellular gene promoters. Finally, comparison of CTIP2-sensititive with P-TEFb-sensitive genes revealed that CTIP2 controls the global P-TEFb function.

Our results suggest that CTIP2 has a double impact on HIV-1 latency. By recruitment of chromatin-modifying enzymes, it favors the establishment of latency; and by inhibiting P-TEFb function, it favors the persistence of latent proviruses. CTIP2 acts directly on the provirus by impacting the HIV-1 promoter activity and indirectly by impacting the gene expression profile of the latently-infected reservoirs.

49.

Interferon-inducible Mx1 protein inhibits influenza virus by interfering with functional viral ribonucleoprotein complex assembly

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Mx1 is a GTPase that is part of the antiviral response induced by type I and type III interferons in the infected host. It inhibits influenza virus infection by blocking viral transcription and replication, but the molecular mechanism is not known. Polymerase basic protein 2 (PB2) and nucleoprotein (NP) were suggested to be the possible target of Mx1, but a direct interaction between Mx1 and any of the viral proteins has not been reported. We investigated the interplay between Mx1, NP and PB2 to identify the mechanism of Mx1's antiviral activity. We found that Mx1 inhibits the PB2–NP interaction, and the strength of this inhibition correlated with a decrease in viral polymerase activity. Inhibition of the PB2–NP interaction is an active process requiring enzymatically active Mx1. We also demonstrate that Mx1 interacts with the viral proteins NP and PB2, which indicates that Mx1 protein has a direct effect on the viral ribonucleoprotein complex. In a minireplicon system, avian-like NP from swine virus isolates was more sensitive to inhibition by murine Mx1 than NP from human influenza A virus isolates. Likewise, murine Mx1 displaced avian NP from the viral ribonucleoprotein complex more easily than human NP. The stronger resistance of the A/H1N1 pandemic 2009 virus against Mx1 also correlated with reduced inhibition of the PB2–NP interaction. Our findings support a model in which Mx1 interacts with the influenza ribonucleoprotein complex and interferes with its assembly by disturbing the PB2–NP interaction.

50.

Type I IFN is produced by HIV-1 infected primary CD4+ T cells

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Background

Production of type 1 interferon (IFN) in response to viral infection requires the detection of viral nucleic acids or proteins by a cellular pattern recognition receptor. HIV-1 is capable of inducing an elaborate IFN response in plasmacytoid dendritic cells (pDCs) through Toll-like receptor mediated recognition of the entering viral RNA genome. However in T cells and macrophages, the main HIV target cells, IFN induction by HIV-1 is considered to be weak or undetectable. Here, we re-evaluate the occurrence of an innate immune response upon HIV-1 infection of primary $CD4_+$ T cells (PBLs).

Methods and Results

Type 1 IFN induction was evaluated in activated purified PBLs during productive infection with HIV-1. In cells from several donors we observed a clear increase in IFNβ and IFNα mRNA levels, as well as induction of several interferon stimulated genes (ISGs) upon HIV-1 infection. The levels of induction progressed concurrently with the levels of HIV infection in the culture. Secreted type 1 IFN protein biological activity was furthermore detected in supernatants of HIV-1 infected cultures. To rule out residual contaminating pDCs as the source of IFN production in these cultures, we performed additional depletion of these cells from $CD4_+$ T cell populations prior to infection and found that type 1 IFN inducion also occurred in the absence of pDCs. Furthermore, we evaluated the biological relevance of the detected levels of type 1 IFN by addition of neutralizing antibodies to IFNβ and IFNα during infection. In presence of the both antibodies, we observed an increase in HIV-1 infection, indicating that the levels of HIV-induced IFN in the T cell cultures are sufficient to have an antiviral effect. To gain more insight in the mechanism of IFN induction by HIV-1, we used inhibitors of reverse transcription, integration and of the HIV-1 protease during single-cycle infection of VSV-pseudotyped HIV-1. We found that integration of the HIV provirus is required for full IFN induction in PBLs, indicating that newly expressed HIV RNA or newly produced HIV proteins are important for evoking an innate immune response in T cells.

Conclusion

These data show that activated PBLs are capable of producing relevant levels of type 1 IFN in response to HIV-1 infection and suggest recognition of newly expressed HIV RNA or proteins as a main trigger of the innate response in these cells. Characterization of the responsible innate immune pathway and pattern recognition receptors will be subject of further investigation.

51.

PSEUDORABIES VIRUS ISOLATES ORIGINATING FROM BELGIAN DOMESTIC PIGS AND WILD BOAR: GENETIC CHARACTERIZATION AND EVALUATION OF THEIR IN VITRO VIRULENCE

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Aujeszky's disease is an economical important disease in domestic swine caused by the porcine alphaherpesvirus, pseudorabies virus (PRV). As a result of large scale vaccination programs, the disease has successfully been eradicated in domestic pigs in a large part of Europe, including Belgium. Serological studies however show that the virus is still present in the wild boar population and the limited literature suggests a reduced virulence of these wild boar strains.

To get first insights in the variability and virulence of strains circulating in Belgian wild boar, five PRV strains isolated between 2007 and 2011 originating from wild boar were genetically characterized by BamHI restriction fragment length polymorphism (RFLP) analysis and phylogenetic analysis based on the sequence of a ± 800 bp fragment of the gC gene. The in vitro virulence of wild boar strains was evaluated by one-step growth curves to determine the in vitro growth capacity and plaque reduction assays to evaluate the sensitivity to interferon (IFN) and was compared with strains isolated from Belgian domestic swine between 1973 and 1989 and the virulent NIA3 strain.

Genetic characterization by BamHI RFLP analysis showed that all wild boar isolates and three of the oldest domestic swine isolates exhibited a restriction pattern type I, typically found for wild boar strains and older PRV strains. The other domestic pig strains where characterized as type II, which is commonly found in European domestic pigs. Based on phylogenic analysis, four of the wild boar isolates are identical apart from one nucleotide substitution and belonged to clade B. The other wild boar isolate and all domestic pig isolates belong to clade A.

Furthermore, one step growth kinetics on continuous swine testical (ST) cells and primary porcine cells revealed no obvious differences in the in vitro growth capacity between isolates from wild boar and domestic pigs. Also, no differences in sensitivity to interferon alpha and gamma were observed between the different PRV isolates on ST cells.

Although genetic differences between wild boar strains and recent domestic pig strains can be observed by RLFP analysis and phylogenetic analysis, this doesn't result in a difference in in vitro virulence. The intrinsic replication capacity and sensitivity to interferon alpha and gamma of wild boar isolates is not different from domestic pig isolates and therefore, when reduced in vivo replication is assumed, other virulence determinants, such as immune evasion mechanisms might be hampered.

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52.

Glycoprotein 150 promotes sexual transmission of Murid herpesvirus 4.

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Gammaherpesviruses are important pathogens in both human and animal populations. During co-evolution with their hosts, these viruses have developed many strategies allowing them to establish latency, reactivate and then shed infectious particles in presence of immune response. Understanding how they do this and what counter-measures might be taken is likely to be important in achieving infection control. We are using Murid herpesvirus 4 (MuHV-4) in mice as a model for the human Epstein-Barr virus and Kaposi's sarcoma associated herpesvirus. Interestingly, we recently observed that MuHV-4 could be sexually transmitted between mice. This model offers therefore the opportunity to test the importance of various immune evasion mechanisms for long-term transmission of these viruses from immune hosts. One of these mechanisms relies on the glycoprotein 150 (gp150) which seems to be used by MuHV-4 as an immunogenic decoy that could limit virus neutralization and promote transmission. Indeed, while gp150 is the commonest glycoprotein target of antibodies raised against MuHV-4, anti-gp150 monoclonal antibodies are not neutralizing but instead enhance Fc-receptor dependent infection *in vitro*. In this study, we tested therefore the importance of gp150 in the context of MuHV-4 sexual transmission. Briefly, we firstly generated a MuHV-4 strain deficient for the expression of gp150 that expresses the firefly luciferase (MuHV-4 gp150- Luc). Female mice were then infected intranasally with wild-type or gp150- MuHV-4 strains expressing Luc and were imaged with an *in vivo* imaging system to follow the infection. Infected females were finally mated with naïve males at the moment of lytic replication in the genital tract to compare capacity of sexual transmission of the two viruses. Our preliminary results show that the gp150- Luc strain has no deficit in infection and in genital excretion compared to the wild type virus. On the opposite, sexual transmission seems to be less efficient for the gp150- Luc strain. In the future, these results will have to be confirmed and we will try to elucidate the molecular mechanisms by which gp150 promotes MuHV-4 transmission.

53.

GENOMIC ANALYSIS OF BELGIAN G2P[4] ROTAVIRUSES REVEALS FREQUENT REASSORTMENT WITH ANIMAL DS-1-LIKE ROTAVIRUSES

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Rotarix™ was introduced in the Belgian national immunization program in 2006, resulting in an almost immediate decrease in the prevalence of hospitalized cases due to rotavirus gastroenteritis. Besides this, a remarkable shift in the rotavirus genotype distribution was also observed: the prevalence of G2P[4] genotypes was low before vaccine introduction (<20%), while after vaccine introduction G2P[4] was the most prevalent genotype in six out of seven rotavirus seasons. Although selective vaccine pressure plays an important role in the increased proportion of G2P[4] strains, genetic changes resulting in an increased virulence or transmissibility of the virus cannot be ruled out as a factor influencing the observed changes in the genotype distribution.

In this study we investigated to what extent changes in the genotype distribution in Belgium after vaccine introduction can also be attributed to genetic changes in circulating G2P[4] viruses. For this we sequenced the NSP4 gene of a representative selection of Belgian G2P[4] strains (n=110) detected in the period 1999-2013, covering both pre- and post-vaccination periods. Subsequently full genome sequencing was applied to a subset of these. All NSP4 genes of the analyzed G2P[4] strains were DS-1-like, but were found in 7 separate clusters of which 6 were more closely related to animal than to human rotavirus strains. Interestingly, the NSP4 genes that clustered more closely to animal DS-1-like strains were all isolated after 2004-2005 and were found throughout multiple seasons indicating a prolonged circulation in humans. Currently, the genomes of 11 strains have been sequenced. All showed the following DS-1-like genotype constellation: G2-P[4]-I2-R2-C2-M2-A2-N2-T2-E2-H2/H3. For NSP5 two out of 11 strains possessed an H3 genotype. In addition, for several G2P[4] strains additional gene segments (VP1, VP2, VP3, VP6, NSP2 and/or NSP5) also clustered more closely to animal than to human DS-1-like strains. These results could indicate that besides the vaccine introduction also an altered genetic composition of G2P[4] strains could have an influence on the increased spread of G2P[4] rotaviruses in the post-vaccination period.

54.

Defining and targeting the HTLV-1 Tax and PDZ proteins interactome.

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Abstract

Primate T-lymphotropic virus species comprise four members (HTLV-1 to -4) that have been discovered in human. Only the HTLV-1 infection leads to adult T-cell leukemia/lymphoma (ATLL). All the four viruses share a similar genomic organization and encode transforming Tax oncoproteins. In contrast to HTLV-2 and 4, HTLV-1 and 3 Tax proteins contain a PSD- 95/Drosophila Discs Large/Zona Occludens-I (PDZ) binding motif at their C-terminal that has been shown to play crucial roles in the distinct transforming properties of the Tax proteins.

Here, we used a collection of human full-length protein-coding open reading frames (ORFeome v3.1) to identify novel PDZ domain containing proteins that specifically interact with HTLV-1 Tax. Novel Tax interactors include syntenin-1 and -2, LNX2, DVL3, GIPC2, INTU, PDLIM4 and -7, RADIL and RGS3. These proteins are involved in diverse biological processes including cell division, cell fate determination and cell suvival. We further characterized interaction between Tax and syntenins and showed that, FJ9 a small molecule able to disrupt Tax/PDZ interactions, could antagonize Tax-transformation activity in rat-1 model.

Our study identify novel PDZ-containg proteins interacting with HTLV-1 Tax and provides the first example where Tax protein-protein interactions whith PDZ-containing proteins and Tax-transformation capacity could be inhibited by a small molecule.

55.

Small-molecule Selective Inhibitors of Nuclear Export (SINE) potently suppress HIV replication

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Many viruses exploit the cellular nuclear-cytoplasmic transport machinery to transport their RNA and specific viral proteins in and out of the nucleus. The karyopherin exportin 1 (XPO1, CRM1) is the major mammalian nuclear export protein and is an essential cellular cofactor for the replication of HIV as it transports the viral late mRNA species from the nucleus to the cytoplasm. The viral protein Rev plays a key role in this transport as it multimerizes on the viral mRNA and interacts with XPO1 through its nuclear export signal. XPO1 on its turn guides the Rev-mRNA complex to the cytoplasm where the complex is dissociated and the viral late mRNAs can serve as templates for viral structural protein synthesis or as viral genome. The interaction of Rev with XPO1 is essential for viral replication and therefore a candidate target for therapeutic anti-HIV strategies. We have designed a class of drug-like, orally bioavailable, small-molecule Selective Inhibitors of Nuclear Export (SINE) that inhibit the Rev-XPO1 protein-protein interaction. We found that SINE trap Rev in the nucleus of the cell and inhibit the nuclear export of the HIV late mRNA species. SINE suppress replication of both wild-type and drug-resistant HIV-1 strains at nanomolar concentration with minimal toxicity to primary cells. Our findings demonstrate that the nuclear export of HIV RNA can be targeted by small-molecules and open new perspectives for the treatment of other viruses using XPO1 as cofactor for their replication.

56.

Broad-spectrum anti-HIV activity of a llama single domain intrabody targeting Rev multimerization

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Rev is an essential viral protein for HIV replication as it ensures export of partially and unspliced viral RNA from the nucleus to the cytoplasm. Therefore, it multimerizes on a secondary RNA structure, the Rev Responsive Element (RRE) present in every partially and unspliced viral RNA. Using the cellular karyopherin CRM1, these viral RNA's are then exported to the cytoplasm for translation and/or packaging.
Recently we discovered a llama single domain intrabody (Nb190) that targets the head multimerization domain of Rev. Based on the defined epitope of this intrabody, we could now predict and verify activity against different HIV-clades. The two primary amino acids in Rev important for binding of Nb190 are K20 and Y23. Analysis of consensus sequences of different clades revealed that these are highly conserved. Analysis of the secondary amino acids V16, H53 and L60 in the Rev epitope shows that L60 is conserved in every clade while V16 and H53 show some variability. To investigate the antiviral activity of Nb190, we produced C8166 and U87CD4X4R5 cell lines stably expressing Nb190. Infection of these cells with HIV-1_{IIIb} revealed that Nb190 exhibits a 100 to 1000-fold inhibition on virus replication. A similar inhibitory profile was observed with clinical isolates from different clades except for clade F. The latter could be explained by a double mutation in the epitope at amino acids V16 and H53. We next looked at the conservation of Rev residues K20 and Y23 among patient samples. Here we found again that these amino acids are highly conserved while in less than 10% an asparagine and arginine or histidine respectively was present. Mutational analysis of these amino acids into a wild-type Rev background revealed that Nb190 kept its inhibitory effect on the mutants K20R and Y23H but loses its effect when K20 is mutated to an asparagine. However this K20N mutant is strongly affected in its ability to replicate. All together these findings indicate that Nb190 has a broad-spectrum anti-HIV activity.

57.

Rational design of an attenuated recombinant vaccine against Cyprinid Herpesvirus 3 using BAC cloning mutagenesis and in vivo imaging system

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Cyprinid herpesvirus 3 (CyHV-3) is the causative agent of a lethal disease in common and koi carp. Since its emergence, in the late 1990s, CyHV-3 has caused severe economic losses worldwide creating a need for a safe and efficacious vaccine. Taking advantage of the recent cloning of CyHV-3 genome as an infectious bacterial artificial chromosome (BAC), we produced recombinant candidate vaccine strains by deletion of single gene. While producing such a recombinant for ORF134, we unexpectedly obtained a clone deleted for ORF56 and ORF57 in addition to ORF134 (as a consequence of an illegitimate recombination while removing the BAC cassette). Interestingly, this triple deleted recombinant replicated efficiently in vitro, exhibited an attenuated profile in vivo and induced an immune protection against a lethal challenge. To confirm that the triple ORF56-ORF57-ORF134 deletion was indeed responsible for the phenotype observed and to determine the contribution of ORF134 deletion in the attenuation; a double ORF56-ORF57 deleted recombinant and an independent triple ORF56-ORF57-ORF134 recombinant were produced and tested in vivo. These experiments revealed that ORF134 deletion did neither contribute significantly to the attenuation observed nor influence the immune protection induced by the infection. Importantly, these experiments demonstrated that ORF56-ORF57 deletion was responsible for the attenuation observed. Next, to determine the contribution of each locus to the observed attenuated phenotype; two single deleted recombinants were produced for ORF56 and ORF57. In vivo testing of these recombinants suggested that most, if not all, the attenuation observed for the ORF56-ORF57 double deleted recombinant is due to ORF57 deletion. Based on its safety-efficacy profile, the ORF56-ORF57 double deleted recombinant was selected as a candidate vaccine. Its tropism and replication were studied using a derived recombinant strain expressing luciferase and in vivo imaging system.

58.

Checkpoints modulation by the human T-lymphotropic virus type 1 Tax protein

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HTLV-1 is responsible for two main diseases, Adult T-cell Leukemia/Lymphoma and HTLV-1 Associated Myelopathy/Tropical Spastic Paraparesis, for which there is currently no satisfactory treatment. Among the proteins encoded by HTLV-1, Tax appears to play an important role in the mechanisms leading to pathogenicity. We are interested in the mechanisms of cell transformation by Tax and more particularly in the interplay between the viral Tax oncoprotein and the DNA damage response (DDR). We demonstrated that transient expression of Tax results in DNA damage, cell cycle arrest and activation of the DDR. In fibroblasts, cell cycle arrest occurs at the G1 and G2 phases depending on the p53 background. Although Tax induces apoptosis and senescence in fibroblasts, HTLV-1 infected lymphocytes proliferate continuously and appear to be adapted to the checkpoint control. This mechanism allows infected lymphocytes to proliferate despite the presence of genomic lesions. Based on these observations, we propose a novel therapeutic approach based on the principle of synthetic lethality.

59.

Viral particles produced in presence of LEDGINs are impaired for infectivity

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Background: Current HIV-1 integrase (IN) inhibitors target the catalytic activity of the viral enzyme IN, which is vital for HIV-1 replication and persistent viral infection. IN mediates the critical step of viral DNA integration. Efficient provirus formation though depends on a crucial cellular co-factor of HIV-1 IN, lens epithelium-derived growth factor (LEDGF/p75), responsible for the tethering of the viral DNA to the host chromatin. It has recently been demonstrated that LEDGINs, small molecules designed to bind to the LEDGF/p75 interaction site of HIV-1 IN and to disrupt the interaction between IN and LEDGF/p75, potently block HIV replication in cell culture through a multimodal mechanism of action. Next to blocking early replication (integration), LEDGINs also affect the infectivity of progeny virions.
Methods: We have now analyzed the late effect of LEDGINs in detail using multiple complementary techniques: (1) electron microscopy to study the morphology of viral particles, (2) virological profiling and preintegration complex (PIC) visualization in infected cells to pinpoint the antiviral effect of LEDGINs and (3) biophysical methods such as FRET to study the effect of LEDGINs on IN multimerization in mature viral particles.
Results: LEDGINs significantly reduce the infectivity of newly produced HIV-1 particles in different cells including primary cells and do not interfere with nascent particles assembly, maturation or RNA packaging. LEDGINs did not reduce infectivity of LEDGIN-resistant HIV strains demonstrating that the effect is mediated specifically through their interaction with IN at the LEDGF/p75 bind pocket of IN and not a unrelated off target effect. Western blot analysis revealed the incorporation of LEDGF/p75 in viral particles which is inhibited in the presence of LEDGINs. FRET analysis demonstrates that LEDGINs enhance IN multimerization in the virions supporting a multimodal mechanism of action targeting IN. Q-PCR and fluorescent PIC analyses demonstrate that HIV particles produced in presence of LEDGINs are impaired for post-entry early replication events including reverse transcription, and nuclear import in the next round of infection.
Conclusion: Next to inhibiting integration, LEDGINs impair the infectivity of viral particles through an integrase dependent mechanism. The combined early and late

effects define LEDGINs as a unique class of antiretrovirals with clinical potential for prevention and first-in-line therapy.

60.

Towards a DNA-based live-attenuated vaccine against the yellow fever virus and other flaviviruses

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The yellow fever virus (YFV), a mosquito-borne flavivirus, causes severe and life-threatening infections with jaundice, systemic bleeding, shock and multi-organ failure. 900 million people living in 45 endemic countries of Africa and Latin America are at high risk. Although there is a safe and highly efficient live-attenuated prophylactic vaccine [YFV-17D, Stamaril®] available, an estimated 200,000 cases of yellow fever occur each year resulting into ~30,000 deaths. Vaccination with Stamaril® is recommended for everybody living in and traveling to the high risk regions. Prompt detection and rapid response through emergency vaccination campaigns are essential to control yellow fever outbreaks (often in remote and resource poor areas). However, the need for a proper cold-chain complicates the timely delivery of vaccines. An easy and inexpensive to produce DNA vaccine would allow much faster and simplified deployment.

We developed a novel reverse genetic system, using an easily tractable multi-host shuttle vector system, for convenient launching of recombinant flavivirus genomes. This system allows to by-pass almost all the problems regularly encountered during cloning, maintenance and manipulation of unstable viral cDNAs. Moreover, the system allows to produce clonal viruses directly from easily scalable plasmid DNAs. We demonstrated that the particular characteristics of the YFV-17D thus generated are identical to that of the original vaccine virus (regarding replication efficiency, virus yield and plaque phenotype). Moreover, when this naked YFV-17D plasmid DNA was injected in AG129 mice, it resulted in the same pathology, morbidity and mortality as the parent virus. The induction of neutralizing and protective immunity is being and will be demonstrated employing a wild-type YFV hamster challenge model. In fact, hamsters transfected with our YFV-17D DNA vaccine seroconvert and mount YFV antibody titers that are comparable to those induced by Stamaril®.

The convenient, robust and reproducible system presented here may allow to develop a DNA vaccine for YFV at low costs without the need for eukaryotic cell cultures or embryonated chicken eggs. It will no longer require a cold-chain and might be needle-free administered. Moreover, our technology may represent a convenient platform for future engineering and manufacturing of other recombinant live-attenuated and chimeric flavivirus vaccines.

61.

Bromodomain and extra-terminal (BET) proteins target Moloney Murine Leukemia Virus integration to transcription start sites

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A hallmark of retroviral replication is stable integration of the viral genome in the host cell DNA. This characteristic makes retroviral-derived vector particles attractive vehicles for gene therapy. However, retroviral integration is not a random process. Lentiviruses preferentially integrate in the body of active transcription units, while gammaretroviruses, including Moloney Murine Leukemia Virus (MLV), favour transcription start sites and CpG islands. In clinical trials using gammaretroviral vectors for gene therapy, leukemogenesis has been associated with integration of vectors near oncogene transcription start sites. We found that the bromodomain and extra-terminal (BET) proteins (BRD2, BRD3 and BRD4) interact with MLV integrase and direct integration towards transcription start regions. BET proteins specifically bind and co-localize with the gammaretrovirus integrase protein in the nucleus of the cell. The interaction is gammaretroviral specific and mediated by the integrase C-terminal domain and the BET extraterminal (ET) domain as determined by co-immunoprecipitation assays and in an Alphascreen assay using recombinant proteins. Interfering with chromatin interaction of BET proteins via specific bromodomain inhibitors JQ1 and I-BET decreases MLV virus replication and MLV vector transduction 5- to 10-fold, while HIV vector transduction is not affected. Analysis of viral DNA intermediates by quantitative PCR revealed a block at the integration step. In addition, bromodomain inhibitors do not have an effect on the late steps of viral replication. MLV integration site distribution analysis revealed a strong correlation with the BET protein chromatin binding profile. Finally, expression of an artificial fusion protein that merges the BET integrase binding domain with the chromatin interaction domain of the lentiviral targeting factor LEDGF/p75, retargets MLV integration into the body of actively transcribed genes, paralleling the Human Immunodeficiency Virus (HIV) integration pattern. Our results explain the molecular mechanism behind gammaretroviral integration site targeting and suggest methods for carrying out gammaretroviral vector transduction with a safer integration site profile.

62.

Ribavirin inhibits in vitro hepatitis E virus replication through depletion of cellular GTP pools and is moderately synergistic with interferon-alpha

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Hepatitis E virus (HEV) is a common cause of acute hepatitis that results in high mortality in pregnant women and may establish chronic infections in immune-compromised patients. We have demonstrated for the first time that ribavirin and interferon-α inhibit in vitro HEV replication, both in a subgenomic replicon and an infectious culture system based on a genotype 3 strain. Interferon-α showed a moderate but significant synergism with ribavirin. These findings corroborate the reported clinical effectiveness of both drugs. In addition, the antiviral activity of ribavirin against wild-type genotype 1, 2 and 3 strains was confirmed by immunofluorescent staining. Furthermore, the in vitro activity of ribavirin depends on depletion of intracellular GTP pools, as is evident from the fact that (i) other GTP-depleting agents (EICAR and mycophenolic acid) inhibit viral replication, (ii) exogenously added guanosine reverses the antiviral effects and (iii) a strong correlation ($R^2 = 0.9998$) exists between the antiviral activity and GTP depletion of ribavirin and other GTP-depleting agents.

63.

Active surveillance of swine influenza virus: potential role of porcine oral fluid samples

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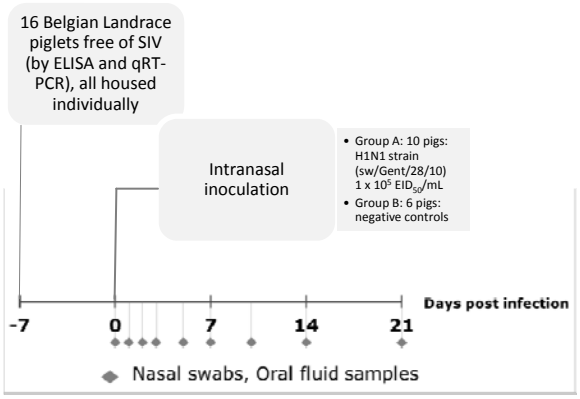
Introduction

The lack of seasonality of swine influenza virus (SIV) infection (1) in combination with the role of swine as "mixing vessels" and the ongoing infections of humans with pH1N1 reassortant H3N2 influenza virus at county fairs, stress the importance of SIV surveillance in order to proactively address the pandemic potential of swine influenza. To date, active surveillance of SIV worldwide is barely done because of the short detection period in nasal swab samples (2). Therefore, more sensitive and reliable diagnostic methods to monitor circulating virus strains are requisite.

Materials and Methods



qRT-PCR was performed using an M gene-targeted RT-PCR procedure. Briefly, all samples were

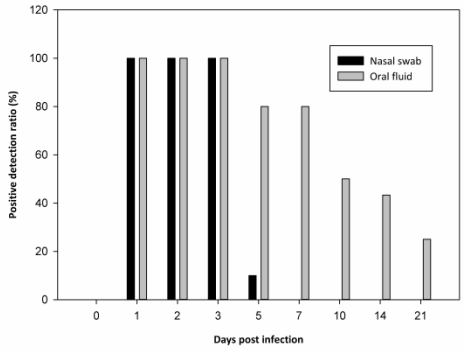
- Extracted: MagMAX Pathogen RNA/DNA kit (Life Technologies)
- Amplified: Vetmax Gold SIV detection kit (Life Technologies).
- Run on a LightCycler 480 Real-time PCR system (Roche).

Virus isolation was performed using embryonated chicken eggs (ECE) and Madin-Darby canine kidney (MDCK) cells according to procedures previously described (OIE Terrestrial Manual 2008).

Results

SIV was detected with qRT-PCR for a longer time period and with a higher rate of detection in oral fluid samples. Especially noteworthy was the possibility to detect swine influenza virus at 21 dpi in 25% of the oral fluid samples, while all nasal swab samples were already negative at 7 dpi.

Fig.1. Positive detection ratios from nasal swab samples and oral fluid samples of sw/Gent/28/10 (H1N1) inoculated pigs



Although comparable amounts of SIV were shed in oral fluid and nasal swab samples, when ECE and MDCK-cells were compared for their ability to detect SIV in oral fluid and nasal swab specimens, it was found that nasal swabs remain the preferred sample over oral fluid for VI and that VI is more effective with cell culture than with ECE.

Discussion and conclusion

Information obtained from surveillance activities could play a pivotal role, not only for pandemic risk assessment but also for laboratory diagnostics, vaccines and antiviral susceptibility. Although a variety of specimens are suitable within a surveillance system, nasal swabs are the most commonly used samples for the diagnosis of SIV. Even so, our results show that the collection of oral fluid samples could represent an alternative, sensitive method for the diagnosis, monitoring and surveillance of SIV in pig populations.

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64.

Study of the mechanism by which a novel PI4KIII inhibitor and enviroxime inhibit HCV replication.

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Several groups reported that PI4KIIIα is an important host factor for HCV replication. PI4KIIIα creates a micro-environment of phosphatidyl-4 phosphate (PI4P) lipids at the viral replication complexes. Knockdown of PI4KIIIα expression drastically changed the membranous web morphology and disturbed viral RNA replication (Reiss et al, 2011). Furthermore, PI4KIIIα seems to be involved in NS5A phosphorylation. Similarly, PI4KIIIβ activity is required for replication of picornaviruses. Inhibition of PI4KIIIβ by enviroxime and enviroxime-like compounds was shown to inhibit picornavirus replication (Arita et al, 2011). Interestingly, we recently demonstrated that enviroxime also inhibits HCV replication *in vitro*. We here report the identification of BF738735, a novel inhibitor of *in vitro* HCV genotype 1a and 1b subgenomic replicon replication. BF738735 was designated as an enviroxime-like inhibitor, since it also inhibits picornaviruses and selects for the same resistance mutations in poliovirus as enviroxime. In enzymatic assays both BF738735 and enviroxime inhibit PI4KIIIβ 10-fold more potently than PI4KIIIα. The protein and mRNA expression of both lipid kinases in replicon containing cells are not affected upon treatment. Interestingly, only siRNA knockdown of PI4KIIIα resulted in inhibition of HCV replication, whereas knockdown of PI4KIIIβ did not. Short-term treatment changed the PI4P pattern/levels in the plasma membrane. To further characterize the mechanism of action of both compounds, BF738735-resistant and enviroxime-resistant replicons were generated (fold resistance=80 and 69). The BF738735-resistant and enviroxime-resistant replicons are cross-resistant with each other and with AL-9 (PI4KIIIα inhibitor) and PIK93 (PI4KIIIα/β inhibitor). The intracellular PI4P levels and PI4P localization in the resistant replicon cells are similar to wild-type. When RNA of resistant replicon cells is stably transfected in Huh 7 cells in the presence of low concentrations of compound, the susceptibility to enviroxime decreased 10 to 20-fold, suggesting that (at least) part of the resistant phenotype is conferred by adaptations in the viral genome. Two mutations in NS4B (V38M, D167E) and several in NS5A were identified in the resistant replicon population. Swapping of NS5A of the resistant replicons or introduction of the V38M mutation into a wild-type genome did not result in a transfer of the resistant phenotype. Replicon carrying the D167E mutation had a pronounced reduction in replication fitness as compared to wild-type and could therefore not be assayed for susceptibility to enviroxime. Studies to further unravel the mechanism of resistance to BF738735 and enviroxime are still ongoing.

65.

2-Hydroxyisoquinoline-1,3(2*H*,4*H*)-diones (HIDs), novel inhibitors of HIV integrase with a high barrier to resistance

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Clinical HIV-1 integrase (IN) strand transfer inhibitors (INSTIs) potently inhibit viral replication with a dramatic drop in viral load. However, the emergence of resistance to these drugs underscores the need to develop next-generation IN catalytic site inhibitors with improved resistance profiles. Here, we present a novel candidate IN inhibitor; MB-76, a 2-hydroxyisoquinoline-1,3(2*H*,4*H*)-dione (HID) derivative. MB-76 potently blocks HIV integration and is active against a panel of wild-type as well as raltegravir-resistant HIV-1 variants. The lack of cross-resistance with other INSTIs and the absence of resistance selection in cell culture indicate the potential of HID derivatives compared to previous INSTIs. A crystal structure of MB-76 bound to the wild-type prototype foamy virus intasome reveals an overall binding mode similar to that of INSTIs. Its compact scaffold displays all three Mg^{2+} chelating oxygen atoms from a single ring, ensuring that the only direct contacts with IN are the invariant P214 and Q215 residues of PFV IN (P145 and Q146 for HIV-1 IN respectively) which may partially explain the difficulty of selecting replicating resistant variants. Moreover, the extended, dolutegravir-like linker connecting the MB-76 metal chelating core and p-fluorobenzyl group can provide additional flexibility in the perturbed active sites of raltegravir-resistant INs. The compound identified represents a potential candidate for further (pre)clinical development as next-generation HIV IN catalytic site inhibitor.

66.

Feline Herpesvirus Ocular Disease
Developing an Antiviral Ophthalmic Solution for Cats

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Feline herpesvirus 1 (FHV-1), a member of the *Alphaherpesvirinae*, is one of the most common viral pathogens of domestic cats and the causative agent of feline viral rhinotracheitis. In addition, FHV-1 has a predilection for the corneal epithelium resulting in an array of ocular disease manifestations and on occasion even blindness in cats. Although antiviral therapy has become standard practice in the management of human herpesvirus infections, no specific anti-herpes drug has been licensed for veterinary application. Here, we report on the pre-clinical and clinical development of a nucleoside analogue into a specific ophthalmic solution for the treatment of FHV-1 ocular disease in cats. The active substance displayed an *in vitro* anti-FHV-1 activity superior to aciclovir, ganciclovir, penciclovir and cidofovir. This nucleoside analogue is activated by the viral thymidine kinase and the 5'-triphosphate is a selective inhibitor of the herpesvirus polymerase. A series of local tolerance, pharmacokinetic and dose-determination and dose-confirmation studies led to the selection of a safe, well-tolerated and effective formulation. The established dosing regimen of 0.2% w/v ophthalmic solution administered three times daily by topical instillation of a single drop per eye per application is currently being confirmed in a pivotal randomised, placebo-controlled, blinded, multi-centric in-use safety and effectiveness field trial in cats with naturally occurring FHV-1 ocular disease.

67.

Comparison HIV-1 Envelope DNA vaccine candidates within three different animal models: Guinea Pigs, Rabbits and Cynomolgus Macaques

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Background

We have selected and optimized HIV-1 DNA vaccine candidates encoding HIV-1 envelope (clade B) proteins by systematic testing in rabbits and guinea pigs for induction of broadly neutralizing antibodies (bNAb). Subsequently, the optimal candidate (SSImix) in the rabbit model was used for immunization of cynomolgus macaques and the immune responses within the three animal models compared.

Methods

This mixture of 3 Env codon optimized gp140 plasmids was used for intradermal immunization at week 0, 4, 8 and 12 of either New Zealand white rabbits, Dunkin Hartley guinea pigs or cynomolgus macaques, four animals each. Sera collected at weeks 0, 2, 4, 8, 12 and 14 were screened in gp120-IIIB ELISA and serum or IgG was analyzed for neutralizing antibody (NAb) response in the pseudovirus TZMbl assay.

Results

Evaluation of gp120-specific IgG in immunized animals demonstrated a response already after the initial priming immunizations. However, the antibody titers did not increase with the same magnitude. At week 14 the highest response was obtained in rabbits, while responses in both guinea pigs and macaques were similar but (~2 log) lower. Neutralizing capacity of antisera obtained from immunized animals clearly differed between animal models used. The highest and broadest NAb responses were found in guinea pigs followed by rabbits while only poor responses were found in macaques (Kruskal-Wallis, $p = 0.0308$).

Conclusion

Parallel immunizations using the same DNA construct in 3 different animal models induced specific antibody responses in all animals, but the neutralizing activity was dramatically lower in the cynomolgus macaques. This suggests species-specific differences in the quality of immune response to HIV-1 env DNA and emphasizes the importance of choosing the correct animal model when screening for future vaccine constructs.

68.

Inhibition of Chikungunya virus replication by T-705 (favipiravir) and identification of resistance associated mutations in the RNA-dependent RNA polymerase

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Chikungunya virus (CHIKV) is a mosquito-borne, emerging human pathogen that causes a debilitating, often persistent arthralgia. T-705 (favipiravir), a nucleotide prodrug, is in development for the treatment of influenza and has demonstrated antiviral activity against different RNA viruses. Here, we describe the antiviral activity of T-705 on CHIKV replication and provide proof that a mutation in the RNA-dependent RNA polymerase (RdRp) gene is responsible for phenotypic resistance to the compound.

T-705 was found to inhibit the *in vitro* replication of a panel of laboratory strains and clinical isolates of CHIKV with EC_{50}s in the range of 2-25 μM (CC_{50} >600 μM). The compound was next evaluated in AG129 mice infected with CHIKV strain S27. Oral administration of T-705 (300 mg/kg/day) reduced virus-induced mortality by 85% and 65% when given pre- or post-infection respectively. Three CHIKV strains, selected independently in the presence of T-705, proved ~2-fold less susceptible to the antiviral activity of the compound. Mutation K291R was observed in the RdRp gene of all three strains. Reverse-engineering of this mutation substantiated its association with T-705 resistance. When the strains were further passaged with the compound additional mutations were selected and a further decrease in susceptibility was observed (~9-fold). A possible association between these additional mutations and resistance is being investigated.

To our knowledge this is the first report showing that T-705 resistance can be selected.

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69.

Discovery and development of a novel class of highly potent pan-serotype inhibitors of dengue virus replication that target NS4b

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The four serotypes of dengue virus have an extraordinary high global public health impact with about 360 million people annually becoming infected, roughly 96 million patients develop disease of which more than 1 million suffer severe disease such as hemorrhagic fever. There is neither a vaccine, nor antiviral drugs available for treatment or prophylaxis. Following a large CPE-based screening effort [employing, a highly diverse small compound library] and extensive hit-to-lead optimization, we identified a novel class of highly potent inhibitors of *in vitro* DENV2 replication. The compounds exert pan-serotype activity with EC_{50} values in the low nM range obtained for DENV1 to 4 (both lab and clinical strains) and > 4 $\log_{10}$ reduction of viral RNA yield or, in most cases, reduction to undetectable levels (as determined by RT-qPCR). The antiviral activity is observed in multiple cell types (Vero, hepatoma, A549, primary dendritic, and mosquito cells). No activity is observed against (un)related RNA viruses such as the hepatitis C, the Chikungunya and the respiratory syncytial virus nor DNA viruses (HSV1); some analogues exhibit modest activity against the related yellow fever virus. This class of DENV inhibitors has a promising ADME-tox and PK- profile. In DENV2-infected AG129 mice, a marked protective effect against virus-induced disease was observed. The compounds inhibit viral replication *in vitro* at a time-point that coincides with the onset of intracellular viral RNA synthesis. Drug-resistant variants were only detected following 15 to 30 weeks of culturing in suboptimal concentrations of the inhibitors and resistant viruses are compromised in their replication fitness. Resistance-conferring mutations map to the NS4b protein. Cross-resistance is readily observed within the compound family but not to other NS4b targeting compounds i.e. lycorine (Zhou *et al*., Virology 2006) and NITD-618 (Xie *et al*., J Virol 2011) arguing for a unique molecular mechanism of action. In conclusion, we have identified a class of highly potent and pan-serotype inhibitors of DENV replication that target NS4b.

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70.

Synergistic activation of HIV-1 expression by compounds targeting the positive transcription elongation factor b (P-TEFb) and by inducers of the NF-κB signaling pathway.

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Objectives : The persistence of transcriptionally silent but replication-competent HIV-1 reservoirs in patients under cART is considered as a major hurdle to virus eradication. Various strategies to purge these residual reservoirs are therefore needed. Bromodomain and Extraterminal (BET) inhibitors (JQ1, I-BET, I-BET151) alone or in combination with Prostratin have recently been identified as compounds able to reactivate HIV-1 from latency. This activation is dependent on the positive transcription elongation factor b (P-TEFb). Moreover, the dependency on Tat viral protein was investigated and still remains unclear.

Here, we investigated the reactivation potential of various P-TEFb inducers (HMBA, JQ1, I-BET, I-BET151) alone or in combination with two NF-κB signaling pathway activators (Prostratin and Bryostatin-1) in several latency model cell lines and in cART-treated patients.

Methods : p24 ELISA assays, RT-qPCR, FACS, cytotoxicity tests, transfection and chromatin immunoprecipitation assays. The reactivation tests were carried out in cultures of $CD8^+$ depleted PBMCs or in $HLA\text{-}DR^-$ cultures isolated from blood of HIV-1 cART-treated individuals with undetectable viral load.

Results : We found that HMBA, JQ1, I-BET and I-BET151 increased HIV-1 production in a dose-dependent manner in latently-infected J-Lat T cells and in U1 promonocytic cell line with minimal cytotoxicity. Next, we tested reactivation potential of P-TEFb inducers in combination with Prostratin/Bryostatin-1. We observed that co-treatment led to strong synergistic activation of HIV-1 production in two latency cellular models. Flow cytometry experiments revealed that the combination P-TEFb inducer + NF-κB inducer

Currently, we are performing experiments to assess the mechanistic insights concerning Tat dependency in reactivation using latent J-Lat cells lines A2 (+Tat) or A72 (-Tat) and transient transfection assays. We are also evaluating the physiological relevance of this synergism in *ex-vivo* cultures of HLA-DR^- CD4+ T cells from cART-treated patients.

Conclusion: Our results suggest the administration of PTEF-b inducers combined with PKC activators together with continuous cART as potential strategies to reactivate HIV-1 from latency.

71.

A Michael acceptor inhibitor of the 3C protease with broad spectrum anti rhinoviral activity

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SG85, a peptidic α,β-unsaturated ethyl ester, was developed during a structure based design of Michael acceptor inhibitors of the enterovirus protease 3C (2). Next to a potent activity against the purified 3C protease of enterovirus 68, enterovirus 71 poliovirus and echovirus 11 were inhibited in a live virus cell based assay. Furthermore, the compound SG85 inhibits a broad spectrum of different rhinovirus serotypes, including both rhinovirus A and rhinovirus B strains or minor and major receptor group strains. The 50% effective concentration (EC_{50}) activity of this compound against human rhinovirus 14 (HRV14) is 77 ± 41nM. A drug-resistant HRV14 virus isolate was selected for SG85 and shows, compared to the wild-type virus and as measured by MTS, a >4-fold increase in resistance to the antiviral effect SG85. Genotyping drug-resistant HRV14 revealed a double amino acid mutation in the non-structural 3C protease (residues S127G and T143A). HRV14 resistant to rupintrivir, another well-known enterovirus 3C protease inhibitor, also contained an amino acid mutation at residue T143 of the protease (1). Reverse engineering of single and double amino acid mutations into an infectious clone of HRV14 revealed no change in the antiviral activity of SG85 for the single mutations 3C S127G or 3C T143A. However, the double mutant HRV14 3C S127G T143A was three times less sensitive to SG85 compared to wild type HRV14.

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72.

Preventing the spread of Foot-and-Mouth Disease virus with antiviral drugs

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After the 2001 Foot-and-Mouth Disease (FMD) epizootic, in which more than 4 million uninfected animals were preventively culled, the EU changed its policy in favor of emergency vaccination (European Council Directive 2003/85/EC). FMD emergency vaccines are serotype- and subtype-specific and confer protection from 7 days post vaccination onwards. Antiviral drugs are serotype-independent and active from the first administration. Antiviral drugs thus provide an attractive tool to bridge the post-vaccinal immunity gap. In terms of international trade restrictions, it might even be more favorable to apply antiviral treatment without emergency vaccination in a treat-to-live scenario, as the post outbreak sero-surveillance phase is presumed to have a shorter duration than when the animals are vaccinated.
Since a number of years, the research consortium of CODA-CERVA, Okapi Sciences and the Rega Institute is developing antiviral drugs against FMD virus (FMDV). Initially, semi-automated high-throughput screening assays were put in place and extensively validated (Willems et al., 2011). More than 65,000 small chemical molecules with drug-like properties were screened for in vitro activity against FMDV. This resulted in the detection of 43 confirmed hit compounds. From these confirmed hits, 21 compound families with a favorable chemical structure and a favorable IP situation were selected for hit exploration and testing of analogue molecules. Three different compound families were selected for lead optimization using a structure-based approach. Significant progress has been made concerning broad-spectrum activity in the low nanomolar range, improved stability and solubility and easy bulk synthesis. This should result in a low cost of goods (10 € /dose). In the next years, hit-to-lead optimization will be continued. A particular emphasis will be put on the identification of the antiviral mechanism at the level of the viral non-structural proteins through the progressive generation and deep sequencing of antiviral resistant mutant FMDV strains. For this purpose, high-throughput next generation sequencing techniques have been optimized and validated (Rosseel et al., unpublished data). A sub-genomic replicon system has been developed to confirm the molecular target under BSL2 circumstances (Murao et al., unpublished data). Optimized highly active compounds with a high barrier for resistance development will be tested for safety and pharmacokinetic properties in rodents and target animals. The in vivo activity will be firstly evaluated in rodents. For this purpose, proof-of-concepts of antiviral activity have recently been shown for the 3-oxo-3,4-dihyro-2-pyrazincarboxamide derivative T-1105 in guinea pigs (De Vleeschauwer et al., submitted) and for the nucleoside analogue 2'-C-methylcytidine in severe combined immunodeficient SCID mice (Lefebvre et al., 2013). T-1105 and 2'CMC are however not potent enough to allow an economically viable control strategy for FMD. Proof-of-concept studies with antiviral drugs in rodents will allow the selection of drug candidates for which a suitable formulation will be developed for administration to target species. The experimental data in natural hosts, together with

epidemiological modeling data, will pave the way for field trials and the final drug development trajectory.

73.

Bovine herpesvirus 4 glycoprotein L is a major target of antibody neutralization

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The neutralization of enveloped virions typically involves blocking cell binding or membrane fusion. The core fusion machinery of mammalian herpesviruses comprises glycoproteins B, H and L (gB, gH and gL). In this study, in order to determine how gL contributes to the viral *in vivo* cycle and how this protein is important for neutralization, we disrupted its coding sequence in Bovine herpesvirus-4 (BoHV-4). While a lack of gL had no impact on the establishment and maintenance of BoHV-4 latency, sera directed against gL deficient virions neutralized significantly less WT and revertant virions than sera directed against WT virions. This difference of neutralization is not caused by a difference in the amount of BoHV-4-specific antibodies present in the sera or by a different response generated against gB and gH. These results suggest therefore that gL-dependent epitopes are the main targets for neutralization of BoHV-4 virions. As one of the functions of gL in BoHV-4 is to trigger virion endocytosis during viral entry, gL-dependent neutralization could affect endocytosis. In the future, unraveling the mechanism of gL-dependent neutralization should help us to develop new strategies against gammaherpesviruses.

74

ROTAVIRUS GENOTYPE DISTRIBUTION IN BELGIUM: CONTINUED HIGH PREVALENCE OF G2, SEVEN YEARS AFTER VACCINE INTRODUCTION

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In 2006 and 2007 respectively, Rotarix™ and RotaTeq™ were introduced in the national immunization program of Belgium. This has led to a dramatic decline in rotavirus gastroenteritis in children below 5. Although both vaccines are available in Belgium, Rotarix™ is by far the most used. For this study we determined the rotavirus genotype distribution in Belgium during the 2012-2013 season.

Rotavirus positive samples have been genotyped at the Gasthuisberg university hospital (GUH) since 1999. Since the 2007-2008 rotavirus season also a national rotavirus surveillance system has been implemented, collecting approximately 500 rotavirus positive samples each season. Rotavirus positive samples were G- and P-genotyped by partially sequencing VP7 and VP4.

The percentage rotavirus positive cases of all patients hospitalized with gastroenteritis at the GUH in 2012-2013 was 7.3%, which is in line with previous seasons. The peak of the rotavirus season was delayed for one or two months when compared to seasons before vaccine introduction (February to March/April). In the first 6 seasons after vaccine introduction G2 was the most prevalent genotype, except for the 2010-2011 season. Preliminary data for the 2012-2013 rotavirus season show that the most prevalent genotype in Belgium was G2P[4] (64.8%), followed by G3P[8] (14.4%), G1P[8] (8.3%), G9P[8] (6.8%) and G4P[8] (4.9%). In addition, we also found one G3P[14] strain, which is a rotavirus genotype typically found in rabbits.

The rotavirus incidence at the GUH strongly decreased from approximately 20% before vaccine introduction to 5-7% since 2008-2009. However, the G2 genotype was the most prevalent in six out of seven rotavirus seasons after vaccine introduction. In all rotavirus seasons after vaccine introduction the relative proportion of the G2 genotype was higher than in seasons before vaccine introduction, most likely due to selective vaccine pressure. However, there is still fluctuation in the genotype distribution indicating that other factors besides vaccination also play a role.

75.

Eradication of persistent bovine viral diarrhea infection in cell culture by antiviral treatment: How to get ahead of the viral evasion strategy.

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The bovine viral diarrhea virus (BVDV) is a member of the family of *Flaviviridae*. BVDV exists as two biotypes, i.e. cytopathogenic (cp) and non-cytopathogenic (ncp). The ncp variant can establish a persistent infection in live stock as well as in cell culture by eluding the host innate immunity. Here we report on how such a persistent BVDV infection can be completely eradicated from mammalian cells. To this end the persistently infected cells were treated for a number of consecutive passages either with different inhibitors or combination thereof. Afterwards the cells were passaged two more times in cell culture medium without inhibitors. For each passage the presence of intracellular and extracellular viral RNA was monitored. An initial experiment resulted in a rapid decline of viral RNA for all inhibitors studied. However, treatment with 10 µM BPIP resulted in a rise in viral RNA around passage 2. To exclude the emergence of drug resistant variants the genomic region coding for the viral polymerase implicated in BPIP resistance was sequenced. Furthermore, the chemical stability of BPIP during treatment was also assessed. Combined these data enabled the design of different drug regimes that resulted in the total eradication of BVDV ncp in cell cultures. The results obtained could help better understanding how viruses establish persistent infections and how persistently infected cells can be cleared from such an infection. The latter can be of value for sanitation of precious cell lines that are contaminated by an ncp BVDV infection. Furthermore it is investigated if prolonged antiviral treatment of persistently infected cells can restore the innate immunity.

76.

A new method to deliver an attenuated vaccine against bovine leukemia virus in herds

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Bovine leukemia virus (BLV) is a delataretrovirus closely related to human T-cell leukemia virus (HTLV). BLV is associated with enzootic bovine leukosis (EBL), the most common neoplastic disease in cattle. Infection by BLV can remain clinically silent or emerges as a persistent lymphocytosis and a leukemia/lymphoma. We have developed an attenuated vaccine that efficiently and persistently protects against BLV infection. The vaccination rationale relies on the deletion of genes required to induce pathogenesis maintaining the integrity of those involved in infectivity. The attenuated strain elicits a strong anti-BLV immune response and does not spread to uninfected sentinels. Passive antibodies are transmitted to newborn calves via maternal colostrum and persist during several months. Nevertheless, the BLV attenuated provirus does not transmit from cows to calves. Vaccinated animals resist a high dose challenge and remain free of wild-type BLV in herd conditions. Finally, we have developed a new method to deliver the vaccine in large herds.

In summary, we have a strategy to deliver a safe recombinant BLV attenuated provirus with impaired transmissibility that efficiently protects against infection in herd conditions.

77

Use of Staby® technology for development and production of DNA vaccines free of antibiotic resistance gene

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The appearance of new viruses and the cost of developing certain vaccines require that new vaccination strategies have to be developed now. DNA vaccination seems to be a particularly promising method. For this application, plasmid DNA is injected into the subject (man or animal). This plasmid DNA encodes an antigen that will be expressed by the cells of the subject. In addition to the antigen, the plasmid also encodes a resistance to an antibiotic which is used during the construction and production steps of the plasmid. However, regulatory agencies (FDA, USDA and EMA) recommend to avoid the use of antibiotics resistance genes. Delphi Genetics developed the Staby® technology to replace the antibiotic-resistance gene by a selection system that relies on two bacterial genes. These genes are small in size (approximately 200 to 300 bases each) and consequently encode two small proteins. They are naturally present in the genomes of bacteria and on plasmids. The technology is already used successfully for production of recombinant proteins to achieve higher yields and without the need of antibiotics. In the field of DNA vaccines, we have now the first data validating the innocuousness of this Staby® technology for eukaryotic cells and the feasibility of an industrial production of an antibiotic-free DNA vaccine. Moreover, as a proof of concept, mice have been successfully vaccinated with our antibiotic-free DNA vaccine against a deadly disease, the pseudorabies (induced by Suid herpesvirus-1).

78.

2'-*C*-methylcytidine efficiently prevents norovirus-induced diarrhea and mortality in a mouse model

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Human noroviruses are a major cause of foodborne illness, accountable for 50% of all-etiologies outbreaks of acute gastroenteritis (both in developing and developed countries). Norovirus outbreaks are extensive and can disturb the functioning of health institutions, leading to closure of hospital wards and causing life-threatening infections in long-term care facilities. Prolonged and severe disease is less frequent but still relevant among more susceptible groups (elderly, immunocompromised, young children). It is therefore a pressing matter to develop strategies for the treatment and/ or prophylaxis of norovirus infections.

We recently reported the inhibitory effect of 2'-*C*-methylcytidine (2CMC), a hepatitis C virus polymerase inhibitor, on the *in vitro* replication of murine norovirus (MNV). We here evaluate the inhibitory effect of 2CMC on *in vitro* human norovirus replication through a Norwalk replicon model and in a mouse model. For that, alpha/beta and gamma interferon receptors deficient AG129 mice were orally infected with MNV (MNV-1.CW3 strain) and developed severe diarrhea. Survival, weight and fecal consistency were monitored, and viral load in stool and organs quantified. Intestines were examined histologically. 2CMC reduced Norwalk virus replicon replication in a dose-dependent manner and was able to clear cells from their replicon. Treatment of MNV-infected AG129 mice with 2CMC (i) prevented norovirus-induced diarrhea, (ii) markedly delayed appearance and reduced viral RNA titers in the intestine, mesenteric lymph nodes, spleen, lung and stool, (iii) completely prevented virus-induced mortality and (iv) resulted in protective immunity against re-challenge. We demonstrate for the first time that a small molecule inhibitor of norovirus replication protects from virus-induced disease and mortality in a relevant animal model. We also show that MNV is efficiently transmitted from infected animals to non-infected sentinels. This transmission could be efficiently and completely prevented by prophylactic treatment of the sentinels. These findings pave the way for the development of potent and safe antivirals as a prophylaxis and therapy of norovirus infections.

79.

SHe's a novel target for HRSV vaccination.

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Infections with human respiratory syncytial virus (HRSV) occur globally in all age groups. In young infants HRSV is a leading cause of severe lower respiratory tract disease, infant hospitalization and an important cause of death in childhood. Despite decades of research, there is still no vaccine available against the Respiratory Syncytial Virus (RSV). While most subunit vaccine candidates focus at the induction of RSV neutralizing antibodies directed against the major RSV surface proteins F and G, we applied a different strategy. Next to its large immune dominant surface proteins RSV also expresses a third much smaller surface protein, the Small Hydrophobic protein (SH) which is barely immunogenic upon natural infections. We developed a novel RSV vaccine candidate that is based on the extracellular domain of the viral Small Hydrophobic protein (SHe). Immunization of BALB/c mice and cotton rats with the SHe fusion proteins resulted in significant SHe-specific serum IgG titers and reduced lung virus titers following challenge with RSV, as compared to control vaccinated animals. In contrast to immunized mice, SHe-specific IgG could barely be detected in the sera of RSV infected mice and cotton rats or in human reference sera, containing high amounts of RSV neutralizing antibodies. Next to vaccination also passive transfer of SHe-specific immune serum resulted in significant reduction of pulmonary RSV-A replication and associated morbidity. Although SHe vaccination or SHe immune serum can reduce RSV replication in vivo, it fails to neutralize RSV infections in vitro. Passive immunization experiments using wild type and (FcγRI, FcγRIII)$^{-/-}$ BALB/c mice, indicated that the antiviral activity mediated by SHe-specific antibodies strongly depends on Fc Receptors. Moreover, using a conditional cell depletion protocol we could demonstrate that alveolar macrophages play a crucial role in SHe immune serum mediated reduction of viral replication. In contrast, depletion of NK cells by anti-asialoGM1 treatment did not impair the activity of SHe immune serum. Immunostaining revealed that although SHe immune serum could barely detect RSV virions it can readily bind to the surface of RSV infected cells. Taken together, these data suggest that SHe-specific antibodies can reduce RSV replication by macrophage-dependent elimination of RSV infected cells.

80.

TREATMENT WITH ANTI-RABIES VHH PREVENTS OR DELAYS DISEASE AND MORTALITY IN MICE DEPENDING ON THE TIMING OF TREATMENT

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VHH are the smallest functional fragments of heavy chain antibodies occurring in Camelids.[1-3] They are very stable and can be fused into multimeric constructs . Anti-rabies VHH with high affinity for the viral glycoprotein were selected by phage display. Rabies virus is a model neurotropic RNA virus causing an aggressive, lethal infection in mammals. To study the efficacy of potential antiviral therapies in the brain, we developed and validated a highly reproducible brain infection model based on intranasal virus inoculation[4], which is a non-invasive method that preserves the structural integrity of the blood-brain barrier. Upon intranasal virus inoculation, viral RNA can be detected at 1 day post inoculation (DPI) in the olfactory bulbs in some mice and in all mice at 2 DPI. The virus then spreads quickly through of the brain, resulting in an explosion of the viral load in the brain at 4 to 5 DPI, reaching a plateau from 7 DPI, at which time symptoms start. One to two days later, the clinical end point for euthanasia is reached. We have used this model to evaluate the protective efficacy of preventive or post exposure treatment with anti-rabies VHH. In vitro, the monovalent VHH could completely neutralize the virus. Moreover, by combining two VHH with a linker, the neutralizing potency was enhanced to a level equal or superior to that of antibodies[5]. A bispecific combination of two VHH was used for further in vivo work. In mice, preventive administration of low doses of VHH in the nose or brain 1 day before virus challenge completely prevented disease. As was the case for simultaneous administration of virus and VHH in brain, muscle or nose. In contrast, treatment with irrelevant VHH invariably yielded lethal disease. Upon intracerebral inoculation of virus and VHH, viral RNA loads remained negligible in the brain. Post exposure treatment was studied by intraperitoneal injection with VHH 1 day after intranasal virus inoculation. At this stage of the infection, part of the neurons are still uninfected, so treatment with compounds that block virus entry and interneuronal transmission can still be effective. Post-exposure treatment with VHH was able to significantly postpone disease by 1 day, which is comparable to the effect of human anti-rabies immunoglobulins. In conclusion, VHH that recognize the surface glycoprotein of rabies virus can completely neutralize virus infectivity in vitro and in vivo when given prior to or together with the virus inoculum. Treatment after the start of the virus infection, when neurons in the olfactory epithelium and olfactory bulbs have already been infected, can delay the median survival time by 1 day. VHH lack Fc effector functions, but are still able to neutralize rabies virus in vivo. Probably, the protective effect of VHH results from inhibition of virus entry in cells and interruption of the interneuronal virus spread.

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81.

CD4 down-modulating activity of cyclotriazadisulfonamide (CADA) analogs correlates with their anti-HIV potency .

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The CD4 cell surface protein is the primary receptor used by the human immunodeficiency virus (HIV) for the entry and infection of host cells, such as T-helper lymphocytes. Efficient infection of HIV requires a high cellular expression level of CD4, together with the chemokine co-receptors CCR5 or CXCR4. Previous attempts to block the viral access to CD4 by using truncated soluble forms of CD4 as decoy receptor were unsuccessful. However, clinical studies with anti-CD4 monoclonal antibodies (Ibalizumab) showed efficacy in HIV-infected individuals. We have previously reported a class of HIV entry inhibitor compounds called cyclotriazadisulfonamides (CADA). These small synthetic compounds specifically down-modulate the expression level of the CD4 protein on the cell surface and subsequent prevent HIV infection of target cells. Here, we describe how structural variations in CADA derivatives affect the compounds' ability to lower the CD4 expression level and how this correlates with their anti-HIV potency. Seventeen new CADA compounds were synthesized with variations in the sulfonamide chains, triaza ring substituents and ring size. The CD4 down-modulating activity of the compounds was independent of the cell type used: similar relative effects on CD4 were observed for the different analogs in MT-4 lymphoblastoma cells and in a CHO cell line, expressing a CD4-YFP reporter protein ($R = 0.95$, $p < 0.001$). The level of CD4 down-modulation evoked by the compounds and their anti-HIV activity correlated well: the pearson correlation of the comparison between the CD4 expression levels and HIV NL4.3 infection levels in MT-4 cells was 0.92 ($p < 0.001$). The correlation between our different assays confirmed the link between CD4 expression levels and the efficiency of infection by HIV. From our structure-activity data, we concluded that compounds with a high electron density in one of the sulfonamide chains showed the highest potency. Variations in the ring substituents had a minor adverse effect but methylation of a nitrogen atom in the ring caused significant loss of activity. Also, the higher activity of unsymmetrical CADA analogs suggested a bimolecular binding model for the CADA compounds. Remarkably, analogs with a smaller 11-ring backbone retained CD4 down-modulating activity and showed a consistent twofold reduction in compound potency as compared to their 12-ring counterparts. Thus, besides optimization of the sulfonamide side chains, size variations in the essential cyclic backbone are feasible, enabling the future exploration of more variations in triaza ring size to generate more potent CADA analogs as a new class of HIV entry inhibitors.

82.

PROGRESS IN THE FIELD OF MICROBICIDES

Guido Vanham and Kevin Ariën, Institute of Tropical Medicine, Antwerp, on behalf of the European consortium CHAARM (Combined Highly Active Antiretroviral Microbicides)

The Objectives of CHAARM are

1) To develop new antiviral inhibitors as local (vaginal/rectal) HIV prevention
2) To develop microbicide combinations and coformulations
3) To test efficacy of microbicide (combinations) in macaque models of vaginal and rectal challenge.
4) To perform human phase I trial of microbicide combinations.
5) To investigate vaginal biomarkers in microbiota, immune factors and proteome analysis.

With regard to new inhibitors, the focus is mainly on entry, reverse transcriptase (RT) and integrase (INT) inhibitors.

Two classes of entry inhibitors are most advanced:

- CD4 mimetics were developed by Loic Martin (CEA Saclay). They block the interaction between cellular CD4 and the viral envelop (gp120). One of these compounds, M48-U1, formulated in a gel, was able to block vaginal transmission of the recombinant SHIV162 virus in cynomolgous macaques.
- Llama's were immunized with HIV Gp120 or Gp140 and resulting single chain VHH were screened for HIV-neutralizing capacities. A series of broadly neutralizing VHH were identified that recognize either known epitopes (CD4 binding site or CD4i), but also new targets in gp120 and gp41. Some of those VHH were combined as "biheads" with synergistic activity. This work was done by Lucy Rutten and Theo Verrips (Utrecht)

In the field of RT inhibitors:

- A series of novel non-nucleosides (NNRTI) was developed by Koen Augustyns (U Antwerpen). The UAMC 1398 came out as most promising. As compared to Dapivirine, the NNRTI which is in phase 3 clinical trial, UAMC 1398 has a better selectivity index and remains active against Dapivirine-resistant viruses. Pharmacokinetic studies in macaques are in progress.
- A particularly interesting class of RT inhibitors, the "acyclic nucleoside phosphonates" (ANP) is being developed at the REGA institute (Balzarini and Schols). Some of these compounds (e.g. PMEO-DAPym) have a particularly broad spectrum including HIV-1, HIV-2, HSV-1 and HSV-2.

Blocking integration:

- Some novel integrase strand transfer inhibitors (INSTI), developed by Maurizio Botta (Sienna) and Roberto Di Santo (Rome) display activity against mutants, resistant against the first generation INSTI Raltegravir.
- Even more fascinating is the development of potent inhibitors of the crucial interaction between HIV integrase and the cellular cofactor LEDGFp75 by Zeger Debysers group (Leuven). These compounds show no cross-resistance whatsoever with INSTI resistant mutants and may have a higher genetic barrier, because they target a cellular factor.

Besides developing these and other new drugs, efforts are being made to develop innovative co-formulations (e.g. a vaginal ring with the NNRTI Dapivirine and the protease inhibitor Darunavir). The capacity to perform animal studies, phase 1 clinical studies and the search for vaginal biomarkers of safety is also ongoing. The CHAARM collaboration includes not only >

20 European academic centers, but also public-private partnerships (e.g. International Partnership on Microbicides) and companies (e.g. Janssen Infectious Diseases).

83.

Resistance of HCV replication to statins is conferred by cellular changes

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A number of statins, widely used cholesterol lowering drugs inhibiting the rate-limiting step in cholesterol biosynthesis, have been shown to inhibit HCV replication *in vitro*. In HCV infected patients, the addition of statins to the standard of care therapy resulted in increased SVR rates. The mechanism by which statins inhibit HCV replication has not yet been elucidated. In an attempt to unravel the mechanism of anti-HCV activity of statins, fluvastatin (FLV) resistant subgenomic gt1b replicon containing cells were generated by culturing the cells in the presence of increasing concentrations of FLV. The replicon containing cell line thus obtained, proved ~8-fold less susceptible to FLV than the WT cultures (IC_{50}: 22±3 µM versus 2.7±0.5 µM). The FLV-resistant replicon containing cells proved cross-resistant to simvastatin and atorvastatin (12- and 4.1-fold, respectively). Several mutations were identified in the replicon genome, most of which were located in NS5A. To study whether these mutations confer resistance to statins Huh 7 cells were stably transfected with RNA derived from the FLV-resistant cultures. HCV replicon replication in these cultures was as efficiently inhibited by the statins as in cells that had been transfected with WT HCV RNA. Thus mutations in the viral genome are likely not responsible for the drug-resistant phenotype. Then we cleared the FLV-resistant replicon cells from their replicon (using interferon-alfa) and stably transfected them with WT HCV RNA. The resulting replicon cell line proved 4.2-fold less susceptible to FLV (as compared to controls) suggesting that the FLV-resistant phenotype is due to a cellular resistance mechanism. Expression of the efflux transporter P-gp at the plasma membrane was 5.7-fold higher in FLV-resistant replicon cells, as measured by flow cytometry. The increased expression also resulted in an increased functional transport activity as measured by the P-gp-mediated efflux of calcein AM. Studies of the expression levels and functional activity of other statin transporters such as MRP2 are currently ongoing. About 30% of patients taking statins have an insufficient clinical response; the mechanism of this “statin resistance” is not entirely known. Our data suggest that

increased drug efflux may at least be one of the mechanisms responsible for statin resistance in in liver cells.

84.

TARGETING THE DENGUE VIRUS ENVELOPE PROTEIN BY *ESCHERICHIA COLI* K5 DERIVATIVES TO INHIBIT THE INFECTION OF MICROVASCULAR ENDOTHELIAL CELLS

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Dengue virus (DENV), a RNA virus of the *Flaviviridae*, is one of the most important emerging viruses in the world. Every year 50 to 100 million cases of DENV infections are reported. DENV causes dengue fever (DF) and cytokine-mediated alterations in the barrier function of the microvascular endothelium, leading to dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). Dendritic cells, monocytes and macrophages are considered to be the primary targets of DENV *in vivo*, but other cell types, including endothelial cells (ECs), may be infected by DENV as well. We investigated whether DENV infects microvascular ECs and whether such infection can be blocked by inhibition of the interaction between DENV and heparan sulfate proteoglycans (HSPGs).

Using both primary human dermal microvascular endothelial cells (HMVEC-d) and an immortalized cell line (HMEC-1), we studied the interaction between ECs and DENV. Virus infection was evaluated by RT-PCR and flow cytometry. Interaction of the compounds with the viral envelope (E) protein was studied by surface plasmon resonance (SPR) using the receptor-binding domain III of the E protein.

DENV productively infected human dermal microvascular ECs, despite the absence of well-described DENV receptors, such as DC-SIGN or the mannose receptor on the cell surface. However, HSPGs were highly expressed on these cells and pre-treatment of ECs with heparinase II decreased the number of infected cells. Since naturally occurring glycosaminoglycans (GAGs), such as heparin, also reduced DENV infectivity by 90%, our findings suggest that DENV uses HSPGs as attachment receptor on microvascular ECs. The capsular K5 polysaccharide of *Escherichia coli* has the same structure as the biosynthetic precursor of heparin but is devoid of anticoagulant activity. Sulfated *E. coli* K5 derivatives blocked DENV infection of ECs in the nanomolar range. We found that the highly sulfated K5-OS(H) and K5-N,OS(H) inhibited virus attachment and subsequent entry into ECs by interaction with the viral E protein.

Our results indicate that DENV-2 infection of microvascular ECs depends upon the interaction of HSPGs with the viral envelope protein. This interaction can be inhibited by natural GAGs or highly sulfated K5 derivatives. Pharmacokinetic and bioavailability studies should be carried out to determine the potential of these compounds as anti-DENV agents.

85.

REDISTRIBUTION OF HIV-BASED VIRAL VECTORS FOR SAFER GENE THERAPY.

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Retrovirus-based vectors are commonly used as delivery vehicles to correct genetic diseases because of their ability to integrate new sequences stably. However, adverse events in which vector integration activates proto- oncogenes, leading to clonal expansion and leukemogenesis hamper their application. The host cell-encoded LEDGF/p75 binds lentiviral integrase and targets integration to active transcription units. We demonstrated earlier that replacing the LEDGF/p75 chromatin interaction domain with an alternative DNA-binding protein could retarget integration. Here, we show that transient expression of the chimeric protein using mRNA electroporation efficiently redirects lentiviral vector integration in wild-type cells. We then employed this technology in a model for X-linked Chronic Granulomatous Disease using myelomonocytic PLB-985 gp91-/- cells. Following electroporation with mRNA encoding the LEDGF-chimera, the cells were treated with a therapeutic lentivector encoding gp91phox. Integration site analysis revealed retargeted integration away from genes and towards CBX1-binding sites, in regions enriched in marks associated with gene silencing. Nevertheless, gp91phox expression was stable for at least 6 months after electroporation and NADPH-oxidase activity was restored to normal levels as determined by superoxide production. Together, these data provide proof-of-principle that transient expression of engineered LEDGF-chimera can retarget lentivector integration and rescues the disease phenotype in a cell model, opening perspectives for safer gene therapy.

86.

Antiviral agents to control Classical swine fever epidemics: an epidemiologic and economic modelling study in a densely populated livestock area in The Netherlands

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Classical Swine Fever (CSF) represents a continuous threat to pig populations that are free of disease without vaccination. When CSF virus is introduced, the minimal control strategy imposed by the EU is often insufficient to mitigate the epidemic. Additional measures such as pre-emptive culling encounter ethical objections, whereas emergency vaccination leads to prolonged export restrictions. Antiviral agents, however, provide instantaneous protection without inducing an antibody response. The use of antiviral agents to contain CSF epidemics was studied with a model describing within- and between-herd virus transmission. Based on the results of previous transmission studies in pigs, epidemics were simulated in a densely populated livestock area in The Netherlands, with farms of varying sizes and pig types (finishers, piglets and sows).

Our results show that vaccination and/or antiviral treatment in a 2-km radius around an infected herd is more effective than pre-emptive culling in a 1-km radius. The most effective control strategy is to vaccinate animals when allowed (finishers and piglets) and to treat with antiviral agents when vaccination is prohibited (sows).

Using the epidemiological data, a number of strategies were evaluated on their economic merits. Total effects were determined by the size and duration of the outbreak, the control strategy applied and the country/area affected. For a country like The Netherlands, exporting mainly to third countries, the present differences in export ban might be substantial when applying a strategy either with or without a vaccinate-to-live approach. Hence, a control strategy including an antiviral agent is economically viable in case of a CSF outbreak in these settings.

In summary, both from an epidemiological and economic perspective, controlling CSF outbreaks using an antiviral agent seems to be preferred.

87.

Effect of biocides on murine norovirus and feline calicivirus, surrogates of human norovirus

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Human noroviruses (HuNoV) are one of the major agents of human gastroenteritis and the main transmission occurs by faecal-oral route.
The purpose of this work is to test biocide products on surrogate viruses of HuNoV in order to get informations on the viral infectivity and on the integrity of viral genomes.

Two caliciviruses, murine norovirus (MNN) and feline calicivirus (FCV), have been chosen as HuNoV surrogates because presenting comparable structure and physico-chemical properties.
Three commercial biocide products have been chosen (Kenocid 2100®, Virocid®, Alcocid®). The biocide product was tested according to Afnor norm EN 14476. The reduction of viral titer has been calculated and RNA extraction followed by a 1 step RT-qPCR was performed.

Three biocides products tested are able to get a 3 log reduction on the viral titer of surrogates MNV and FCV and are so considered as effective. Efficacy against HuNoV can be extrapolated.
The absence of effect of Alcocid® on genomic copies number indicates this biocide product doesn’t interfere directly on viral genome but it acts maybe only on the viral structure, the capsid for example.
In contrary with the 2 other biocide products (Kenocid 2100® and Virocid®), the increase of Ct values indirectly means an effect on the number of genomic copies. The effect of these two products could be on viral capsid but also directly on viral genome.

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