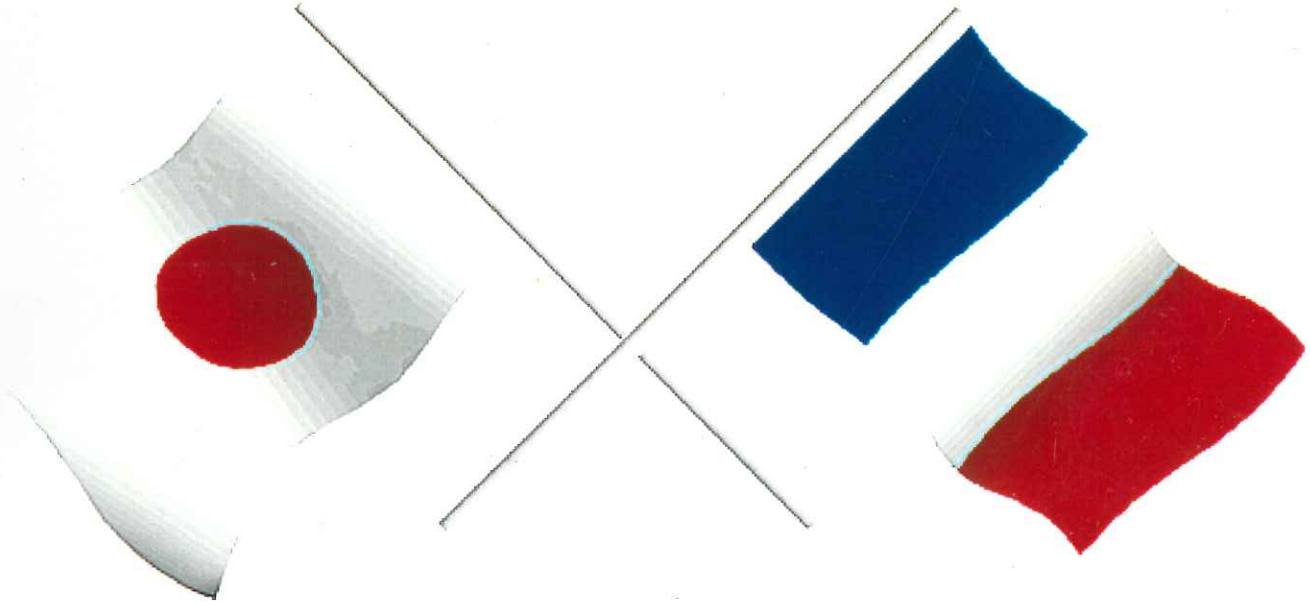
Analysis of the role of viral glycoprotein D in Marek's Disease resistance by somatic and germinal transgenesis

Marielle Afanassieff, Denis Soubieux, Sophie Olayat, Bertrand Pain, Norman Ross, Ginette Dambrine, Pierrick Thoraval



FRENCH - JAPANESE WORKSHOP

'Genes And Early Development'

June 4 - 5 1998

Ecole Normale Supérieure de Lyon
46, allée d'Italie 69364 LYON

'GENES AND EARLY DEVELOPMENT'

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Program

June 3: Welcome Party at IBIS Gerland Hotel 19:30

June 4

8:00 - 8:30 Registration at Ecole Normale Supérieure de Lyon

8:30 - 9:00 Welcome adress

9:00 - 12:30 Session I : Germ Cells, Moderator : Dr B. PAIN

9:00 - 9:30	Tomoni YOSHIMIZU	Mechanisms of germ cell establishment in pre-gastrulation mouse embryo
9:30 - 10:00	Bertrand PAIN	Avian embryonic stem cells : a promissing tool for efficient avian transgenesis
10:00 - 10:30	Takashi KUWANA	Avian germ-line cells in biotechnological aspect
10:30 - 11:00	Coffee Break	
11:00 - 11:30	Masaru TAMURA	Development and sex differentiation of the mouse fetal germ cells and gonads
11:30 - 12:00	Shin-Ichi ABE	Initiation of meiosis from newt spermatogonia in vitro by mammalian FSH
12:00 - 12:30	Discussion	

12:30 - 13:30 Lunch at Ecole Normale Supérieure de Lyon restaurant

13:30 - 16:00 Session II: Cell Differentiation, Moderator : Dr F. DIETERLEN

13:30 - 14:00	Shin-Ichi NISHIKAWA	Cultures for ES cell differentiation: cell specification without body plan?
14:00 - 14:30	Thierry JAFFREDO	Developmental relationships between endothelial and hematopoietic cells analyzed in the avian model
14:30 - 15:00	Estelle HIRSINGER	Role of different factors in chick somitic differentiation
15:00 - 15:30	Sumihare NOJI	Molecular mechanisms for vertebrate limb initiation and specification
15:30 - 16:00	Coffee Break	
16:00 - 18:30	Poster Session	

18:30 - 20:30 Special visit of the old city with a guide

20:30 - 23:30 Dinner in a restaurant

23:30 Return to the hotels by a special bus

June 5

8:30 - 12:30 Session III: Transgenesis, Moderator : Dr L.M. HOUDEBINE

8:30 - 9:00	L.M. HOUDEBINE	The vectors for the expression of transgenes
9:00 - 9:30	Xavier VIGNON	The use of somatic cells in bovine nuclear transfer
9:30 - 10:00	Kazuma TOMIZUKA	Genetic rescue of mice by introduction of a human chromosome fragment
10:00 - 10:30	C. LA BONNARDIERE	Regulation of porcine trophoblastic interferon genes expression during early pregnancy
10:30 - 11:00	Coffee Break	
11:00 - 11:30	Patrick PRUNET	Variegated expression of a Prolactin-LacZ transgene in the pituitary gland from different lines of transgenic rainbow trout
11:30 - 12:00	Pascale DEBEY	Cytoplasmic control of nuclear fate after natural fertilization and nuclear transfer
12:00 - 12:30	M. AFANASSIEFF	Analysis of the role of viral glycoprotein D in Marek's Disease resistance by somatic and germinal transgenesis
12:30 - 13:30	Lunch at Ecole Normale Supérieure de Lyon restaurant	

13:30 - 17:30 Session IV: Early Development, Moderator : Dr J.S. JOLY

13:30 - 14:00	Franck BOURRAT	Morphological and molecular study of morphogenetic events in the optic tectum of the medaka (Oryzias latipes)
14:00 - 14:30	Jean-Stéphane JOLY	Analysis of the functions of homeobox genes during medaka embryonic development
14:30 - 15:00	Hiroyuki TAKEDA	The mesoderm and neural induction in Zebrafish
15:00 - 15:30	Coffee Break	
15:30 - 16:00	Isao MATSUO	Regulation and function of Otx homeobox genes in rostral head development
16:00 - 16:30	Katsuhiko MIKOSHIBA	Molecular dynamic in brain development. Role of IP3, Zic, CR-50 antigen/reelin in early development
16:30 - 17:00	Yuji WATANABE	Antagonistic regulation by SHH and BMP4 in vertebral development
17:00 - 17:30	Discussion and Concluding Remarks	
18:00 - 20:00	Final party at IBIS Gerland Hotel	

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Marek's disease is an economically important disease of chickens caused by a herpesvirus (MDV) and characterized by development of lymphoid tumors in various tissues and organs including nerves, muscles and viscera. Although vaccines have dramatically reduced losses from Marek's disease, genetic resistance, as well as vaccine-induced immunity are essential to minimize the incidence of this disease. Genetic variation in resistance of chickens to Marek's disease has been amply demonstrated (1). Involvement of the major histocompatibility complex B in genetic resistance was first reported in 1967. But mechanisms of genetic resistance to Marek's disease are still unknown. It appears that multiplication of virus in the host is restricted by genetic resistance. Strains of chickens which were resistant to Marek's disease had less virus in their tissues than did strains which were highly susceptible to the disease. However genetic resistance to infection with MDV has not been demonstrated.

The envelope glycoprotein D of herpesviruses is required for virion infectivity and plays an important role in viral entry at the fusion-penetration step. Hamster kidney cell line which constitutively expresses glycoprotein D of herpes simplex virus 1 (HSV-1) is resistant to infection with HSV-1 and HSV-2 (2). The mechanism responsible for this interference is not known, but glycoprotein D could mediate interference either by interacting with some cell surface component required for viral penetration (3), or by altering the cell surface, or by interacting with the virion surface (4). This interference is shared by several herpesviruses like BHV in bovine (5) and PRV in swine (6).

To analyze the glycoprotein D-mediated interference with MDV and the potential role of this interference in Marek's disease resistance, we are currently using three different approaches: the first one in cell culture, the second one *in vivo* by somatic transgenesis and the third one by germinal transgenesis. In the first analysis, the gD gene originated from the Us region of the RB1B strain of MDV was introduced into an avian leukosis virus-based vector (pUTIRES vector) (7). Helper-free vectors were produced from an avian packaging cell line (8) and used to infect chicken embryonic fibroblasts. Glycoprotein D was shown to be expressed in the cytoplasm of infected fibroblasts by western blotting, immunofluorescence and FACS analyses. The effect of glycoprotein D expression is now analyzed by infection of pUTIRESgD-infected fibroblasts and control fibroblasts with RB1B strain of MDV extracted from feather follicules or with C12/130 strain of MDV extracted from fibroblasts. In the second experiment, the gD gene was introduced into a replicative avian retroviral vector (RCAS vector) (9) in transcriptional sense (RCASgDsense) and antisense (RCASgDantisense). Ten-day-old chicken embryos were infected with 10^6 infectious units of RCASgD viruses by injection into the omphalomesenteric vessels. Twelve days after hatching, RCASgD-infected chicks were put together with MDV-shedding chicks. The incidence of Marek's disease among RCASgDsense-infected chicks, RCASgDantisense-infected chicks, control noninfected chicks and MDV-shedding chicks is currently compared. In the third experiment, the gD gene was introduced into a commercial plasmid vector (pIRES1neo; CLONTEC) under the transcriptionnal control of the human cytomegalovirus major immediate early promoter. This vector was transfected into CEC (Chicken Embryonic Cells) cultures (10) and several clones were obtained after selection by G418. These clones will be injected into stage X chicken embryos as previously described (11) in order to obtain germline chimeras and transgenic chickens for MDV glycoprotein D. We will present here the results of these different preliminary analyses and the state of advancement of transgenesis experiments.

(1) Gavora *et al.* 1979 *Comp. Immun. Microbiol. Infect. Dis.* 2:359-371. (2) Campadelli-Fiume *et al.* 1988 *J. Virol.* 62:159-167. (3) Johnson *et al.* 1989 *J. Virol.* 63:819-827. (4) Dean *et al.* 1994 *Virology* 199:67-80. (5) Tikoo *et al.* 1990 *J. Virol.* 64:5132-5142. (6) Petrovskis *et al.* 1988 *J. Virol.* 62:2196-2199. (7) Benchaibi *et al.* 1989 *Virology* 169:15-26. (8) Cosset *et al.* 1990 *J. Virol.* 64:1070-1078. (9) Hughes *et al.* 1987 *J. Virol.* 61:3004-3012. (10) Pain *et al.* 1996 *Development* 122:2339-2348. (11) Thoraval *et al.* 1995 *Poult. Sci.* 73:1897-1905.