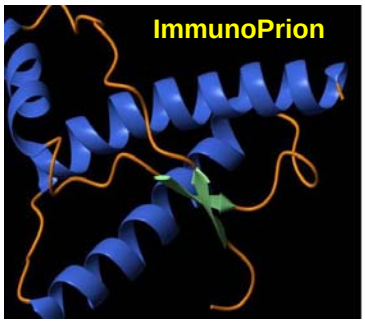

FOOD-CT-2006-023144

IMMUNOPRION

STRAINS, SPECIES AND IMMUNOLOGY IN PRION DISEASES

PUBLISHABLE FINAL ACTIVITY REPORT

Period covered:	**01/06/2008 to 31/11/2009**	Date of preparation:	
Start date of the Project	**01/06/2006**	Duration	**42 months**
Project coordinator:	**Patrice Marche, INSERM**	Revision	**Draft 2**

I- Project execution

The TSEs, such as scrapie in sheep or “mad cow disease” in cattle are thought to occur when an animal (or host) eats material from a diseased animal containing an aggregated, misfolded form of a protein, termed the prion protein (PrP). The misfolded, pathogenic form of the protein is called PrPsc (an abbreviation for PrP (scrapie)). PrPsc survives passage through the stomach, and is absorbed in the intestine, where it makes contacts with cells of the immune system, from which it is eventually passed into cells of the nervous system. During its passage in cells of the new host animal, PrPsc makes contacts with the host animal’s own non-aggregated, physiological PrP (PrPc) protein, and this contact causes the host’s PrPc to aggregate, forming more PrPsc, which causes cell damage and destruction within the nervous system. Many of the events in this chain of reactions are not fully understood, and the aim of the ImmunoPrion consortium was to explore these events further, to achieve explanations, at the molecular level, of the fine details of disease transmission.
One major topic of interest is the transmission of the disease from one animal species to others. This topic is of major importance to the food industry, in terms of the probabilities of transmission to humans. The passage of TSE agents (PrPsc), from one species to another is limited by a so-called “species barrier”. The strength of this barrier varies considerably according to the mammalian species involved and to the characteristics of the prion strains. In some combinations, passage does not appear to occur (for instance hamster 263K scrapie into wild type mice). In others it can occur, but it requires more infectious material and longer incubation periods than transmission between members of a same species. Recent reviews have summarised the current knowledge about prion strain diversity and the molecular mechanisms of the pathogenesis (1-3). Under both natural and laboratory conditions the species barrier is currently unpredictable. No evidence of human contamination by ovine scrapie has been reported so far whereas hundreds of cases of human new variant CJD have been attributed to the BSE (“mad cow disease”) agent. An important parameter in cross species infection resides in the degree of molecular homology between the infectious PrPsc and the host PrP.
Laboratory cell free systems confirm the importance of molecular matching for efficient conversion of PrPc into PrPsc. Yet, such in vitro systems parallel only partially the in vivo reality, suggesting that molecular matching is not the only parameter involved and that additional factors might control the species barrier effect. One of those factors could be the host immune system (both innate and adaptive immunity) which is known to react vigorously against xenogenic PrPc and PrPsc and which, in doing so, could influence the pathogenicity of TSE agents from other species

The ImmunoPrion project addresses three major issues:

- the physico-chemical bases of strain diversity among prion disease agents,
- the structural and physiological parameters that govern the passage of prion disease from one species to another,
- the role of the host immune system, innate and acquired, in TSEs

Main achievements of the project

A. The physico-chemical bases of strain diversity among prion disease agents

1) Mechanism of polymerisation of PrP.

It is likely that the primary structure of the host PrP is one of the key parameters of the observed species barrier. In order to study the molecular process of the conformational transition of PrP into pathological PrPSc, we and others have used recombinant prion proteins (rPrP) to allow experimentation with aggregate forms of different primary structures. Such recombinant prion materials are helpful to define standards for comparison with naturally infectious strains. Simplified in vitro systems were demonstrated to be useful to decipher some molecular parameters. For instance, it was demonstrated that some amino acids (residue 139 and to lesser extend residues 109 and 112) are important in the transmission barrier between hamster and mouse (4), and that fibril formation is intrinsically promiscuous and capable of utilizing heterologous PrP variants as a substrate for polymerization in a highly efficient manner (5).

We set up a series of varied biochemical conditions to obtain amyloid aggregates from rPrP. We followed the kinetics of amyloid formation in various conditions and analysed their conformational features by spectroscopy and microscopy methods. Our work was focused on the establishment of a cell-free system supporting the conformational transition of PrP into PrPsc forms. In parallel, the rPrP and aggregated forms were studied for their biological properties in vitro with cell-free systems and in vivo after inoculation to animals, to serve as antigen source to induce antibody responses in animals.

The characterization (by Flow cytometry, electron microscopy) of amyloids (PrPsc aggregates and fibrils) obtained in vitro demonstrates that they display many different conformations. Changes in buffer composition and/or thermodynamic conditions gave rise to different structures, in the same conditions several structures could be generated (figure 1).

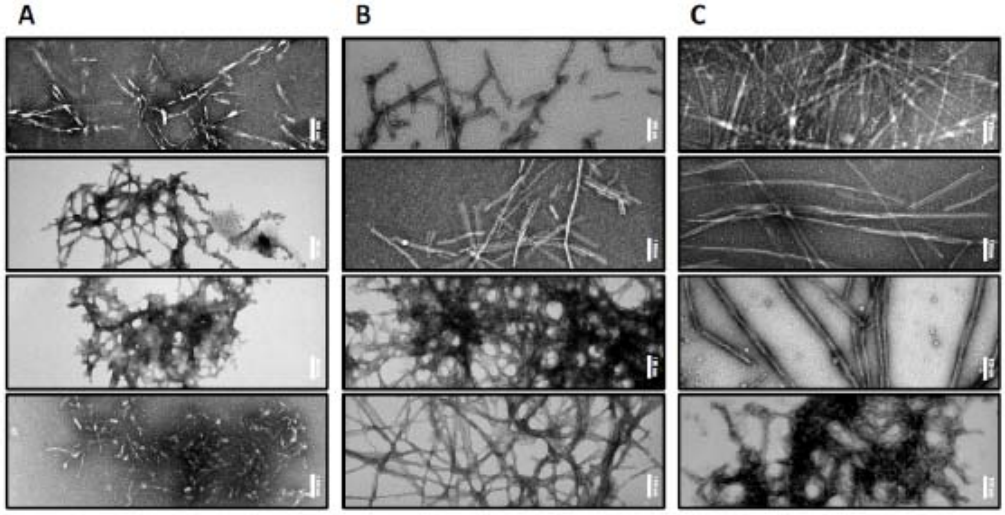

Figure 1. Electron microscopy analysis confirms the heterogeneity of the amyloid structures. Aliquots of samples obtained after polymerization in different buffers (A, B and C) were examined after negative staining by Electron microscopy. The images represent an arbitrary selection among many different structures that were observed during this work. Scale bars represent 100nm. (copyright NKI)

The structures obtained in vitro can support self-replication. The amyloids we have obtained in vitro under these conditions present the characteristics of the strains observed in encephalopathies. From these results we were able to propose a mechanism for the generation of strains (Figure 2).

The PrP aggregates formed in the presence of different denaturing buffers were tested to see if they would “seed” (catalyse) the aggregation of rPrP. SAF (scrapie amyloid fibrils) a natural form of PrPsc purified from the brains of infected hamsters does catalyse such polymerization, but denaturing buffers do not. This means that they are non-infectious, as, if

they were administered to an animal, they would not cause PrPsc formation on contact with the host PrP. Further polymerization conditions, such as non-denaturing, were explored. The resulting aggregate can catalyse polymerization, and therefore is potentially infectious. These materials have been used in animal infection experiments, but because of the long incubation time for development of TSEs, the results will not be known until after the submission of this report.

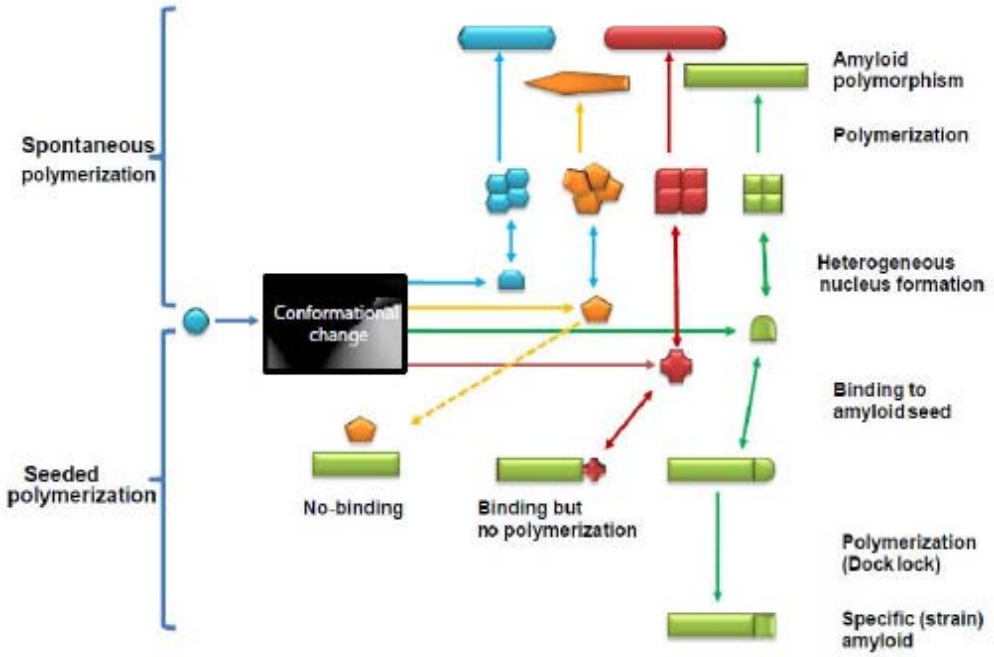

Figure 2. Amyloidogenesis of prion protein in vitro is a nucleation-dependent polymerization process. However, the nucleation is not the main determinant of the lag time, and another mechanism should be postulated to explain the weak dependency to the initial concentration of monomers and the residual lag time observed during seeded polymerization. This mechanism is symbolized by a black box. The diversity of the amyloids obtained with the same purified prion protein implies the genesis of different conformers of the monomer, but only the corresponding conformer can react with a precise amyloid used for seeding. This hypothesis would explain, not only the heterogeneity of amyloids, but also the perpetuation of the strains.

2) The structure of native PrPsc

The description of the structure of the naturally-formed pathologic PrPsc remains a challenging issue. To study the three-dimensional structure of prions close to the native state requires in situ imaging within lympho-reticular cells. We adopted an imaging approach using cryo-tomography with potential for 4 nm resolution, which is not achievable with other methods such as X-ray or NMR. Coupled to tomography analysis, this method yielded structural images in 3 dimensions with 5nm resolution. We succeeded in obtaining vitrified specimens of PrPsc samples, of rPrP and of cells such as follicular dendritic cells (FDC). High resolution images were obtained from PrPSc purified from hamster brains, and proteinase K digested recombinant PrP27-30 samples showing structures with a diameter of 4 +/- 1 nm (Figure 3).

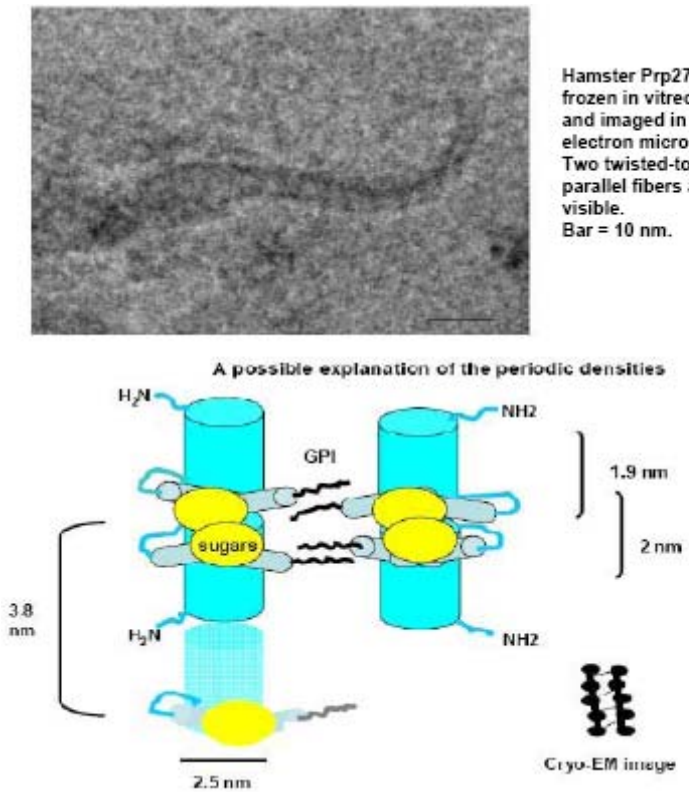

Figure 3. The sample (recombinant hamster PrP27-30) preparation procedure was found to be critical for the integrity of the prion fibrils and to minimize the presence of contaminants. Images of these improved protein samples frozen in vitreous ice were obtained by cryo-ET. These images indicated fibres composed of two or four strands, each with a diameter of ~2 nm. The data did not support models published previously by other groups. Instead, they were consistent with a model in which individual strands are comprised of stacks of monomers. Our current data suggest that this technique cannot yet be used to distinguish prion strains.

This objective is directly related to the strain and species barrier issue since it is expected that formation of different morphological structures of PrP oligomers, aggregates and fibrils may account for the the compatibility or incompatibility between different strains of PrP. This is an important and highly debated issue. Electron microscopy papers with similar objectives have appeared recently, Sim and Caughey (4) used electron and atomic force microscopy to obtain morphological information on different mouse scrapie strains. Basic structural differences were observed between strains such as the diameter and direction of twist and periodicities. These measurements of the amyloid core of scrapie fibrils should aid development of consensus on structural models of prion structure and strain.

To progress further and to image PrPsc in situ in tissues, sensitive methods were successfully developed to detect PrPsc in animal tissues after infection. Detection of prions using electron tomography of vitreous cell or tissue sections remains highly challenging. For this, it was first essential to identify sites where prion fibres are concentrated. Cryo-immunogold electron microscopy was used to investigate the localization of prion protein in murine Peyer's patches during the first three weeks after oral exposure to scrapie prions. PrPsc was detected transiently, one day after ingestion of scrapie material, in large endosomes of follicular associated epithelium (FAE) enterocytes. Subsequently, PrPsc was found in enlarged endosomes of mononuclear phagocytes in the sub-epithelial dome. It then started to accumulate on the surface of follicular dendritic cells, increasing in amount from 7 days up to 21 days after prion exposure to the gut.

B. The structural and physiological parameters that govern the passage of prion disease from one species to another,

1) PrP Structure in relation to antigenicity.

To date, there are no established antibodies able to discriminate PrPSc for monomeric (unaggregated) PrP. We addressed this issue in two ways. The first approach relied on finding, or predicting potential cryptic epitopes of PrP which would be exposed, and thus accessible, in PrPSc. To define such PrP regions, we used in silico molecular modelling, based on Govaerts, Daggett's and Mornon's models (7), and this led to the identification of a region, PrP155-166, which is predicted to be exposed on PrPsc and mostly buried in PrPc. Several independent studies have pinpointed the importance of this region in the conformational transition process. NMR and EPR spectroscopy came to the same conclusion that the region 159-219 converts into a parallel, in-register β-sheet structure (8). Amino acid residues which appear to play key roles include Tyrosine 155 which appears important in the stabilization of the sheep PrP structure, as shown by circular dichroism and NMR spectroscopy (9) and Serine 170 which was shown in PrP from different species (elk, human, mouse and hybrids) to be critical for the plasticity of the structure, by the mean solvent molecular dynamics simulations (10). From the PrP155-166 region, three peptides (B1, B2 and B3) were selected to serve as B cell epitopes. For immunisation attempts, peptides bearing a B cell epitope associated with different T-cell MHC-II restricted epitopes, as helpers for the immune response, were synthesised and were used for immunizations of healthy and prion infected mice. To monitor the immune responses, several methods based on ELISA and flow cytometry were adapted to detect antibodies specific for the small peptides or for Proteinase K resistant PrP proteins (representing PrPsc). Initial results indicated that mice vaccinated with B-T epitope peptides displayed a delay in the pathogenesis induced by the infection with prion inoculums, and that the T epitope from tetanus toxin (830-844) was the most promising in promoting antibody responses towards the selected B cell epitopes of PrPSc. Further consolidatory work showed that no significant difference in immune responses was observed which would allow distinguishing between healthy and prion-infected mice, but the use of certain PrP peptide conjugates proved efficient in delaying scrapie onset and prolonging the clinical stage of the disease. Our results may be considered a basis for future experiments in two possible directions: 1) Development of improved vaccine approaches based on the PrP158-168 region as conformational PrPsc epitope buried inside PrPc, for instance by using more potent carriers (pseudo-virus particles, liposomes...). 2) Search for more pertinent B-cell epitopes by molecular modeling studies. It is possible that the limited clinical effect was due to the fact that raised antibodies mainly targeted fibrillar PrPsc and not the more pathogenic small oligomers.

The second approach was based on the hypothesis that the immune system may control the tolerance towards the different forms of the PrPsc proteins. Recently, several studies (11-13) reported that recombinant peptides may elicit immune response in mice. Therefore, mice displaying defects of the immune system, such as those lacking T lymphocytes, were repeatedly immunised with recombinant PrP as immunogens, and then challenged by infections. But, although the anti-prion reactivity was revealed by a delay in the disease outcomes and by humoral immune responses against PrP protein, there is still no direct evidence of reactivity specific of the PrPSc form.

2) The species barrier explored in vivo in mutant mice.

The cell tropism, ie the cell type with which they become associated, of infectious prion material varies dramatically between animal species. For example, prions are lymphotropic in sheep scrapie and vCJD, but less in bovine spongiform encephalopathy (BSE). This hampers the establishment of accurate animal models for infection of humans by TSEs. We addressed this issue by the development of "humanised" mice (huID) which can mount human immune responses (Figure 4). To control the nature of the host PrP, immunocompromised mice, obtained by knockout of CgammaR, Rag2, were crossed with

PrP knockout mice and then crossed with transgenic mice expressing PrP of different alleles or species. These mice were grafted with CD34+ human cord blood progenitor cells, thus all B-, T-, and NK-cells are of human origin and express a known human PrP. The reconstitutions of the human system were assessed by a detailed analysis of the different organs and cells. The various huID mice can be challenged by infection by various prion materials. Such a reconstitution model offers the opportunity to control precisely different parameters, including aspects of host immune system and host PrP, potentially involved in the control of the species barrier.

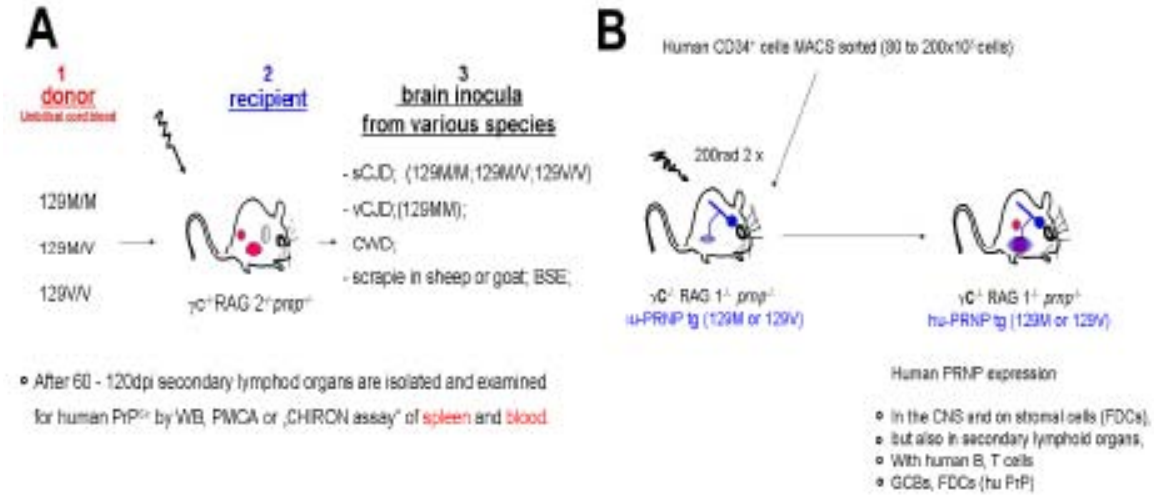

Figure 4. (A) The various genetic polymorphisms of the human PRNP gene (encoded within the purified CD34+ cells) and the various prion inocula that will be tested in humanized mice are indicated. (B) Generation of a humanized mouse that will not only carry human immune cells in secondary lymphoid organs but that will also express the human prion protein in the central and peripheral nervous system.

The huID mice have been inoculated peripherally with prions derived from a human sCJD brain homogenate (129MM). PrPsc appeared in spleen 60 - 90 days post infection. Inoculation with brain homogenates derived from a chronic wasting diseased white tail deer and an elk (CWD) did not yield Prpsc in spleen nor in lymph nodes. We also established humanized mice with PrP polymorphism, by humanization with cells from human donors bearing the PrP 129M/V or 129V/V gene. Again, CWD inoculation did not yield to PrPsc in spleen or in lymph nodes. These data show for the first time that the prions from the emerging infectious chronic wasting disease in the USA are most almost certainly not a risk to humans. Infection experiments with other combinations of prion material and models of Prp transgenic mice will provide further relevant information on the transpecies Prp infections.

C. The role of the host immune system, innate and acquired, in TSEs

A critical process in development of TSE disease involves passage of infectious prion material (PrPsc) from the intestine to neural tissues. Components of the immune system are believed to drive this process. There is growing evidences that PrPsc interacts with projections (dendrites) of dendritic cells (DCs) present in the luminal space of the gut prior to trafficking to lymphoid areas, then infectious prions could expand and spread to neighbouring nerve fibres. Several recent works from the consortium and other groups have provided new insights. In mice, the transient depletion of DC at the time of infection was shown to delay prion replication in the spleen and neuroinvasion, thus increasing the incubation time of the disease (14-17), although this effect remains variable in some instances (18, 19). Gut associated lympoid tissues play a critical role in prion accumulation and neuroinvasion after oral exposure, for instance the number of DC increases in the Peyer's patches (20), and the combined absence of the Peyer's patches or mesenteric lymph nodes prevents PrPSc

transmission (21). Differences in the proportion of splenic FDC in close interactions with sympathetic neural fibres are observed among mice with different incubation periods (22). In cattle, ileal and jejunal Peyer's patches display variations in innervation that might influence the path of neuroinvasion and, thus, the susceptibility to the BSE agent (23). We have evaluated the contribution of status of the host immune system in the permissiveness to infections by hamster passaged prions. Chronic inflammations of the gut were provoked in mice by ingestion of dextran sodium sulphate which induces chronic colitis, and in rats using Indomethacin that induces a rapid, acute inflammation of the ileum and colon. PrPsc multiplies faster in inflamed colon and Peyer's patches (PP) than in non treated mice, and we observed a decrease of host PrP expression in the colon. DSS treatment has no impact upon the progression of the agent from the peritoneal cavity to the CNS. In contrast, disease is retarded in DSS-treated mice infected per os indicating that the neuroretrograde route is dominant when prions enter through the gut. We evaluated the effect of DSS inoculation on sympathetic innervation of the colon, and showed a decrease of sympathetic innervation and that dendritic cells (DC) were less in contact with nerve fibers than in control mice. These data are correlated with a reduction of the neuro-immune interfaces. Gut inflammation by DSS appears to block prion progression probably as a result of a loss of terminal endings.
This observation is consistent with other work and supports the idea that the fate of the disease is determined during the early days which follow oral infection. Experiments in rats offer the possibility to recover immune cells by in vivo cannulation of the nodes. Intestinal inflammation led to accumulation of macrophages and peripheral monocytes in the mesenteric lymph nodes which can be further examined for their PrPsc contents.
The complement system, a major component of innate immunity, is believed to contribute to the localization of prions to FDCs in the early phase of oral contamination. To explore the molecular interactions of prions with complement, we purified several of its components (C1q, C3, C4, factor H) from several species including human, mouse, rat and cow. Using real time molecular interaction analysis, we demonstrated that prion materials bind with high affinity to C1q and factor H (24, 25). These results are reinforced by the observations of other groups showing that various conformational isoforms (native, amyloid fibrils, and beta-oligomers) of recombinant human PrP (90-231 and 121-231) bind to C1q, factor H and C4b (26). Furthermore, the PrP region spanning amino acids 141-159 is proposed as a potential C1q binding site (27). Such molecular complexes between prion material and complement proteins may interact with complement receptors expressed by immune cells, thus providing a bridge between PrPsc recognition in the soluble phase and subsequent association with migrating cells. We analysed prion uptake by DC from rat and mouse by several methods. We found a clear increase of PrP, including PrPsc, by DC when they were exposed to prion materials which were preincubated with serum as a source of complement proteins. However, the PrP content is greatly reduced when prion material is incubated with serum of mice deficient in C1q, thus demonstrating the importance of PrP-C1q complexes in this process. The importance of complement interaction in the species barrier has not yet been studied in detail, but the results of interaction studies between different prion materials and complement proteins from different species show wide cross-reactivity (but different affinities) of C1q of different species with different PrP preparations. Currently the results are in qualitative form, but ongoing quantitative SPR analysis will provide more sophisticated information which will elucidate aspects of cross-species infectivity.
To detect compounds which may interfere with C1q binding to prion material, recombinant segments of C1q, named the A, B and C chain globular heads (28), were shown to inhibit C1q binding. This indicates additionally that all three polypeptide chains of C1q participate in the binding. A low molecular weight compound, betulin disulphate, shown by others (29) to inhibit C1q binding to various targets, also inhibits binding to prion material. Identification of these inhibitors provides a starting point for the design of more potent inhibitors for experimental or eventual medical use.
In conclusion, altogether these works strengthen the evidences that the immune system plays a crucial role in the barrier species in providing new insights and innovative tools to further study the mechanisms of the transmission of prions.

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ImmunoPrion project "Strains, Species and Immunology in Prion Diseases" was contracted by the European Commission (FP6-023144) from June 2006 to November 2009. The project is co-ordinated by Patrice Marche, from INSERM (Fr).
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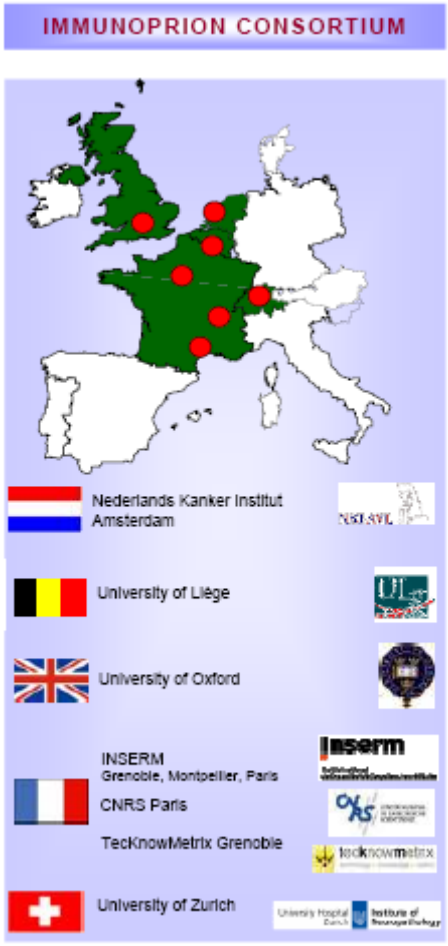
Public web site: http://immunoprion.vitamib.com

ImmunoPrion consortium was formed in response to the call FOOD-2004-T5.4.5.3 of FP6. It is composed of 7 participants who belong to major academic institutions from Belgium (University of Liege), France (Institut National de la Santé et de la Recherche Médicale, Centre National de la Recherche Scientifique), Netherlands (The Lowenhook Cancer Institute), Switzerland (University of Zurich and UK (Oxford University Departments of Pathology and Biochemistry).
These academic partners are experts in a wide range of fields including the pathogenesis of Transmissible Spongiform Encephalopathies (TSEs), Immunology, Neurology, Veterinary medicine and Structural Biology. In addition, the consortium includes a small private company specialized in the management of scientific knowledge and in the correlation of data, patents and trends in specific industrial or academic fields.



II Dissemination and use

Over the lifespan of the project, the partners have communicated the scientific results in the frame of internal project meetings and external meetings or congresses by poster presentations, talks at conferences, networking participations. Finally several peer-reviewed publications have been released (see table below). The ImmunoPrion website is a contact platform linking partners together for internal efficient and rapid exchanges and for external communication with public and research/development actors.

The consortium released a project flyer that has been distributed at all suitable opportunities such as conferences, networking meetings, universities. The flyer is still available and can be ordered beyond the project duration.

IMMUNOPRION OBJECTIVES

The major objective of ImmunoPrion project is

to improve food safety by:

- Investigating the fundamentals of Transmissible Spongiform Encephalopathies (TSE)
- Enabling the rational development of detection and control strategies
- Bringing multi-disciplinary research to the frontier of the current knowledge
- Organizing innovation and discovery transfer to society

To achieve these goals ImmunoPrion addresses three key issues:

- The strain diversity of TSE agents
- The passage of the species barrier
- The evaluation of the host innate and acquired immune responses

These questions correspond to critical interrogations in the field of TSE pathogenesis.

SCIENTIFIC RESEARCH

The passage of infectious prion agents from one species to another is limited by the so-called "species barrier". The species barrier is highly variable, depending on the origin of the strain of prion, the species of the infected host and the route of exposure to the pathogenic agent.

The transfer of pathogenic prion agents between different host species is a major issue for quality control in the food chain. The bewildering diversity of Prion-associated diseases will be analysed using different strains of prions and different routes of infection. This work will impact on the control of the food chain by providing diagnostic procedures and recommendations for safety policy.

WP01-A 3-D imaging approach of prion strains in lympho-reticular cells. The structural basis of "prion strains" will be defined by comparing prions of natural origin with recombinant prion proteins produced *in vitro*. These proteins will be characterised biologically and immunologically.

WP02-In vitro synthesis of prion strains. The structural basis of "strains of Prion" will be defined by comparison of natural origin and of in vitro produced recombinant Prion proteins. Biological and immunological properties of these Prions proteins will be characterised.

WP03-The neuroimmunology of prion diseases. The pathological effects induced by Prion infection via different routes will be studied by physiological analysis of the nervous and immune systems.

WP04-Contribution of the host immune system to the species barrier effect. The role of the host immune system and of intestinal inflammation in the passage of infectious agents from one species to another will be determined.

WP05-Molecular mechanisms that underpin prion trafficking and infectivity.Interactions of prion strains with the innate immune system, including complement proteins and dendritic cells, will be analysed in different host species to determine their impact on the pathogenesis of infection.

WP06-Design of immunization tests based on peptides that mimic PrPsc. The conditions needed to break natural tolerance to pathogenic prions will be defined in order to generate specific humoral and cellular immune responses to prion proteins or peptides and to obtain reagents specific for pathogenic prions.

WP07-Prospective search and information shares with industrials and governing institutions. The results of this work will be disseminated widely in the scientific community, industrials, and policy deciders to be exploited for the benefit of public food safety. Interested partners will be identified with the aim of exploiting knowledge generated by the scientific activities .

Communication towards other actors of Prion

ImmunoPrion partners exchanged with other Prion related European and international projects such as NeuroPrion (EU) and PrionNet (Canada). Members of ImmunoPrion teams participated to events of these consortiums, thus contributing in sharing new knowledge issued from research in TSE and in discussing risk assessment for human and animal health and management in the food industrial process.
Dissemination and communication towards industry and societal actors were further achieved through three dedicated workshops organized by ImmunoPrion consortium with the participation of actors from research/development, industry, unions of animal producers, safety agencies. These workshops addressed specific issues of the field: 1) potential impact of works on industrial process & initiation of first network of industrial partners, 2) foreseen impact on risk assessment and regulation of discoveries in TSE, 3) risk assessment and regulation in TSE & Impact on EC road map.

ImmunoPrion's achievements and exploitable results yield to applications with potential interest for developing, creating or marketing product or process or providing services:

- Generating pathogenic synthetic prions is a challenging issue. Know how has been established to produce *in vitro* replicating Prp from synthetic materials after seeding with amyloids. Such assays may have value to determine the pathogenic potential of samples.
Exploitable result: Prp replicating properties
Exploitation potential: *in vitro* replicating assay for risk assessment for industry. (contact INSERM: liautard@univ-montp2.fr)

- Detection of prions in cells or tissues remains problematic. The development of new imaging methods based on electron tomography of vitreous cell or tissue sections was achieved allowing the detection of the accumulation in experimentally infected tissues..
Exploitable result: imaging of Prp in tissues
Exploitation potential: assay for detection of prion accumulation for risk assessment. (contact NKI: ppeters@nki.nl)

- Interaction of Prp with host counterparts has progressed through the development of methods to measure molecular interactions. Chip system is grafted with panels of proteins of interest (proteins or peptides including Prp) and molecular association is detected by Surface Plasmon Resonance imaging, which allows direct (without label) and parallel measures (in arrays). Such chip system displays potential applications in a wide variety of fields.
Exploitable result: Protein/Peptide Chip
Exploitation potential: system and assay for the measure of protein interactions. (contacts INSERM: patrice.marche@ujf-grenoble.fr ; Uni Oxford: rbsim@bioch.ox.ac.uk)

- Compounds binding to complement components inhibiting interactions with Prions
Exploitable result: Inhibitors of Prp proteins
Exploitation potential: molecules which affect interactions between Prp protein and complement components. (contact Uni Oxford: rbsim@bioch.ox.ac.uk)

Complement components plays a major role in the innate immune system of the host defenses. Due to their relative weak stability their purification for later uses remains in some instance difficult or yet impossible.
Exploitable result: Platform of purification and characterisation of complements proteins
Exploitation potential: system and assay for the measure of protein interactions. (contacts INSERM: patrice.marche@ujf-grenoble.fr ; Uni Oxford: rbsim@bioch.ox.ac.uk)

- Prion infectivity through the species barrier is a major concern in risk assessment and emergence of new pandemy. Sevearl *in vivo* infection models were established through the development of “humanized” mice, harboring a human immune system, and bearing different forms of the Prp gene (from various species or defective Prp genes). Mice can be exposed to prions by different ways (injection, alimentation) and the establishment of disease can be monitored. Thus, the infectivity is established in relation to the species origin of the Prp gene and in the context of a human type immune system.
Exploitable result: Infectivity assay
Exploitation potential: in vivo infectivity assay for risk assessment. (contact Univ Zurich: Mathias.Heikenwaelder@usz.ch . heikenwaelder@helmholtz-muenchen.de)

Definition of Prp structure and potent epitopes. In silico simulations allowed to identify B cell epitopes as potential antibody binding sites. Peptides bearing these epitopes were used in infections to determine their potential benefit in disease protection or progression.
Exploitable result: Vaccine and assays to monitor immune response
Exploitation potential: B-cell epitope in the PrP proteins and assay for to monitor immune response against prion materials. (contact INSERM: pierre.aucouturier@inserm.fr) ;

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Tropismes et barrières d'espèces des ATNC (maladies à prions): Quels dangers pour l'homme ? Jacques-Damien Arnaud, Maria-Teresa Alvarez-Martinez Pascaline Fontes et Jean-Pierre Liautard Rev. Franc Lab. In French (in press)