Project no. FP6 2004 Food 3B 023183

Project acronym:

StrainBarrier

Project title:

Understanding prion strains and species barriers and devising novel diagnostic approaches

Instrument: STREP

Publishable final activity report

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Section 1 – Project Execution

i) Summary description of project objectives

Introduction: The recent epidemic of bovine spongiform encephalopathy (BSE, or mad cow disease) in Europe, its transmission to humans (in the form of variant Creutzfeldt-Jakob disease, vCJD), and the rapid spread of chronic wasting disease (CWD) among deer in North America illustrate the severe public health threat posed by prions.

One of the most unsettling features of TSEs is the existence of stable prion *strains*, which differ in incubation times, clinical features, and neuroanatomical PrP^{Sc} deposition, as well as in epidemiological properties. Strains have added a layer of complexity to the already arduous prion problem. Sheep which were considered genetically resistant to classical scrapie, for instance, succumb to new "atypical" scrapie strains, thus turning the table on previous eradication approaches. The question of whether animal species previously unaffected by prions (such as pigs) will become infected by new strains becomes more relevant than ever. Some strains, including BSE, easily transmit to other species (crossing the "species barrier"), causing serious concerns in the EU. Such "promiscuous" strains could propagate through the food chain, while remaining either undetected or misidentified. There are as yet no diagnostic procedures to identify TSE strains, except in some rare cases.

Prion strains are encoded by alternative PrP^{Sc} folding patterns, but the structural and biological features which encode strains and determine their disease phenotype and transmission properties remain essentially obscure. Prions can be studied at the structural level (how is the protein folded?), biochemically, at the cellular level (how do prions invade the cell and persist in it?), at the animal level (what will be the symptoms, the neuropathology?) and at the level of epidemiology (how do strains transmit from species to species? how do they then manifest clinically? Do they "mutate"?). All these approaches are taken by the StrainBarrier partners. Central to this study are transgenic mice expressing the PrP gene from clinically relevant species (such as human or bovine), which mimic in part the behavior of prions in the corresponding species.

Objectives and approach: The EC research consortium *StrainBarrier* is studying fundamental and applied aspects of prions strains and their relationship to the species barrier, with the goal of providing better tools and methodologies to diagnose prion strains and to predict their behavior.

1. Systematically study field BSE/scrapie strains, their species barriers in transgenic mice expressing sheep, cattle, porcine, murine PrP.
2. Evaluate the permeability of the animal-to-human species barrier of these TSE agents.
3. Validate the use of a transgenic mice panel for a rapid and highly efficient identification and characterization of TSE strain biodiversity.

4. Study fundamental aspects of strains: PrP^{Sc} structure, cell biology and pathogenesis.
5. Develop novel diagnostic and research reagents such as strain-dependent antibodies and cell culture systems.
6. Interact with national disease control agencies.

ii) Contractors:

Participant no.	Participant name	Participant short name	Country
1, Coordinator	Albert Taraboulos	HUJI	Israel
2	Peter J. Peters	NKI-AVL	Netherlands
3	Krister Kristensson	KI	Sweden
4	Jesus R. Requena	USC	Spain
5	Chiara Zurzolo	IP	France
6	Juan Maria Torres	INIA	Spain
7	~~MabGenics GmbH~~	~~MabGenics~~	~~Germany~~
8	Olivier Andreolotti	INRA	France
9	Dolores Gavier-Widen	SVA	Sweden
10	Marion Simmons	DEFRA	UK
11	Carsten D. Korth	HUDUS	Germany

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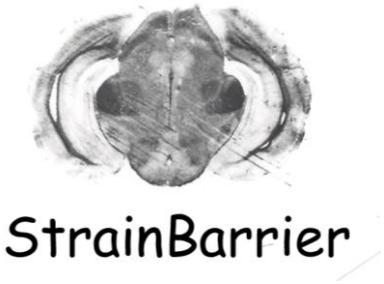
iii) Project website: **http://strainbarrier.huji.ac.il/**

iv) Project logo:



v) Degree to which the objectives have been reached.

The objectives of the StrainBarrier project were to (i) improve our understanding of the prion strain phenomenon at the structural/molecular, cell biological, and epidemiological levels, and (ii) to prepare novel reagents, devise assay system, and use the knowledge generated in order to improve our ability to detect prions and diagnose strains.

These goals were largely attained. In addition, a number of unexpected discoveries were made, and the project has led to a better understanding of the biology of prions.

vi) Work Performed and Principal Results.

The consortium performed the following studies:

1. Structural studies were performed primarily by CR4 Requena and CR3 Peters using purified "prion rods". These partners focused on two stable and well known strains of hamster-adapted scrapie and used a variety of biochemical, biophysical, and electron microscopic techniques to study their conformation. As a result, these partners succeeded in pinpointing minute conformational differences between the PrP^{Sc} structures in these two strains.

2. Prions maintain a parasitic relationship with the host cell. On the one hand, the host cell provides the biological molecules and processes needed for the prion to replicate. On the other hand, prions influence their host and are usually harmful. Examining these aspects of the prion-host interaction yielded a large crop of interesting results. For instance, CR5 Zurzolo discovered that prions can move from cell to cell by using "tunneling nanotubes", microscopic bridges that can often form between neighboring cells. She also identified, for the first time, an endocytic compartment which is involved in the formation of PrP^{Sc}. CR3 Kristensson, on the other hand, established a detailed map of signaling pathways which are involved in the interaction of prions with the host. He also determined that enzymes in the host lysosome can degrade away PrP^{Sc}, and demonstrated that strains differ in their sensitivity to these enzymes.

3. Understanding and diagnosing strains require new reagents. Here too, the StrainBarrier partners developed very important tools. For instance, CR11 Korth developed reagents that can differentiate between prions of different strains. In another study, CR6 Torres and CR5 Zurzolo developed several systems of cultured cells that can be infected with prions, in which the biology of strains can be studied.

4. Crowning these efforts are very extensive studies performed by CR6 Torres and CR8 Andreoletti. These partners used transgenic mice to model the possible behavior of various prion strains in animal species. Their results provide new and very important insight on how strains behave when they jump from one species to the other such as the delay in incubation time, the stability of the strain, etc.

vii) Relation to State of the Art.

The StrainBarrier project set out to (i) generate a better understanding of the nature of strains, (ii) improve existing procedures designed to identify strains and predict their behavior. Many of the results described above are novel and unexpected, and they will help change the scientific landscape in which prion scientists operate.

viii) Impact.

European countries aim at the highest possible standards of consumer protection and for highest possible standards concerning the safety of food and of biological products. The existence of various prion strains differing in incubation times, clinical features, and epidemiological properties has always been disquieting, because the nature of strains was not understood and their properties remained unpredictable. In the last decade, however, the emergence of novel promiscuous and "atypical" strains has added a great degree of urgency to the strain problem. In particular, it became evident that the prion scene is much larger than previously thought. Bovine and ovine prions are a good example of the novel dangers. Classical scrapie of sheep has been known for centuries, and there is no evidence that it has caused disease to humans. Two decades ago, it turned out that scrapie and the new BSE agent likely belong to different strains, and that sheep are susceptible to the new strain and can develop sheep BSE. As BSE is more promiscuous that scrapie, this discovery raised concerns that a covert epidemic could develop in sheep and catch us unprepared. This example illustrates the need for a better understanding of what strains are, how they behave (for instance in terms of species barrier), and how to rapidly identify them.

It is important to keep in mind that, while strains add a degree of complexity to prions, the biology of prions remains poorly understood irrespective of their division into strains. Thus, novel insights about prions are urgently needed as well.

In this setting, the impact of StrainBarrier is likely to be large and manifold.

1. StrainBarrier has provided a host of results about prions *per se*, irrespective of strains. The cell biology of prions appears to have especially benefited from these studies (the first PrP^{Sc}-

synthetic compartment; tunneling nanotupes transport prions from cell-to-cell; signaling networks mapped in prion-infected cells, etc). However, other aspects will benefit too: novel expertise was generated as to the detection of PrP^{Sc} in brain section by cryo-electron microscopy; a novel insight was attained as to the properties of protease-sensitive PrP (CR4 Requena), etc. This new knowledge will help to advance the science of prions.

2. StrainBarrier has generated a very large body of data as to the behavior of strains as they cross species (their new incubation time, their strain stability, the biochemical properties of the strains in the two species, etc). This will help epidemiologists and health authorities to predict possible zoonotics, etc.

3. Finally, reagents (strain-specific PrP^{Sc}-binding peptides, antibodies), powerful cell systems (neurospheres, etc), and methodologies were developed that will help both authorities and scientists in their endeavor in the field of prions. This will have an important impact on public health, food safety, and prion research.

StrainBarrier has resulted in many publications in refereed journals as well as numerous presentations at scientific conferences as well as in the lay media.

2. Publishable results of the plan for using and disseminating the knowledge.

CR11 Korth developed novel strain-specific, PrPSc specific peptides. These reagents are derived from W226, a mouse monoclonal antibody that binds PrP with high affinity. The peptides were described in the following publication:

Müller-Schiffmann A, Petsch B, Leliveld SR, Muyrers J, Salwierz A, Mangels C, Schwarzinger S, Riesner D, Stitz L, Korth C (2009). Complementarity determining regions of a funtional anti-PrP scFv orchestrate conformation-specificity and antiprion activity. Molecular Immunology, 46:532-540.

Possible applications include using these peptides in assays for prions and prion strains, for convcentration of prions out of dilute samples, and for removal of prions from contaminated fluids.

The contact person in connection with these peptides is Dr. Carsten Korth, Department of Neuropathology, Heinrich Heine University Düsseldorf, Moorenstrasses 5, 40225, Düsseldorf, Germany