Proposing T-independent B-cell activation by prion rods: Could disease result from 'chaperoning' of nascent prions by PrPsc-cognate immunoglobulins?

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The mechanism of invasion of the central nervous system (CNS) by prions remains a mystery. B-cells appear to play a crucial role, since intraperitoneal injection of the infectious prion particle fails to cause brain disease in mice lacking B-cells. It was originally supposed that infectious prions probably convert native B-cell-borne prions (PrPc) into the disease-causing infectious form (PrPsc) at a peripheral site, and that this is then followed by conversion of neuronal PrPc into PrPsc by B-cell-borne PrPsc. Though attractive, this scheme has been invalidated by the recent finding that repopulation of B-cell-deficient mice with B-cells from prion(gene)-knockout mice results in just as effective a reversal of the block to neuroinvasion as repopulation with B-cells from normal mice, suggesting that B-cells are crucial but that B-cell-borne prion protein molecules are not. As an alternative, I argue here for the involvement of B-cell-borne antigen receptors, also called surface immunoglobulins (sIg). I propose that prion rods specifically cause thymus-independent activation and proliferation of naive B-cells bearing PrPsc-cognate sIg, through crosslinking of sIg by PrPsc-specific epitopes displayed in repeating fashion on the prion rod. Consequently, proliferated B-cells circulate through various lymphoid organs and tissues, effectively transmitting the structural signal for prion misfolding by functioning as bearers of 'plaster-cast' (sIg) moulds on which PrPsc epitope-defining regions of nascent prions have the opportunity to preferentially adopt localized PrPsc conformation through 'induced-fit' interactions. Such binding is further proposed to cause restriction/chaperoning of the conformational freedom of the remaining chain length of the nascent prion polypeptide, forcing it to fold into the PrPsc conformation. Initial transmission of the misfolding signal through this route is proposed to be followed up by other proposed mechanisms of nucleation-propagation within the nervous system. Some tests are proposed.

Prominent among prion diseases that can begin with infection through a peripheral route are scrapie, kuru, Creutzfeldt-Jakob disease (CJD), bovine spongiform encephalitis (BSE), and new variant CJD (nv-CJD). How prions administered through oral or intraperitoneal routes actually manage to invade the central nervous system is still a mystery[1]. In this paper, I attempt to arrive at an answer that blends critical insights from immunology and protein structural biochemistry. I do this principally as an intellectual exercise, to provoke thought and initiate debate, and do not pretend that the hypothesis that follows provides answers to all questions about prion disease that remain unanswered.

The prion hypothesis: What is already known/believed

Prions are infectious, 'protein only' pathogens. Unlike conventional infectious pathogens, which possess a nucleic acid genome, and propagate within infected hosts through synthetic duplication of their entire chemical makeup, prions are believed to replicate only through propagation of an aggregation-prone conformation among the host's own prion protein molecules[2]. In a manner that is not yet fully understood, prions structured in a pathogenic conformation (PrPsc) are thought to induce the body's 'normal' prions (PrPc) to misfold in their (pathogenic) image. The suggestion that a protein entity lacking a nucleic acid genome might possibly mimic the behaviour of an infectious pathogen was originally met with so much outrage (some of which still persists[3]) that it would be well in order to explain the prion hypothesis here, with a pithy analogy, to avoid misunderstandings about exactly how prions are conceived to replicate. Prion infection is somewhat akin to the 'spoiling' of a class of obedient students by the entry of a new student who is disobedient and unruly. The disobedient student does not replicate to create copies of himself/herself *ab initio*, but only happens to influence some (otherwise obedient) students already present in the classroom, turning them into disobedient students. To the observer sensitive solely to the adverse effects of 'disobedience' in the classroom, the entry of the disobedient student appears to result in the *de novo* creation of hordes of disobedient students. Whatever the precise etiology, increase in the numbers of disobedient students (resulting from the entry of one or more such students) leads both to direct and collateral damage to the class. In the nutshell of an analogy, this is what prion propagation, infectivity and pathology are thought to be all about.

There is much experimental evidence supporting the prion hypothesis. The two conformations, PrPc and PrPsc, have been studied and found to differ in their secondary and tertiary structural content (a region that is helical in PrPc adopts a beta sheet structure in PrPsc)[4,5]. PrPsc has been shown to be independently capable of causing prion disease through both intracerebral and peripheral administration, and it has been established that prion disease cannot occur in an animal lacking the prion gene[6,7]. Prion infection can be established in tissue culture of neuronal cells

through addition of PrPsc[8]. Much of the PrPc conformation stands determined at atomic resolution through 2-D NMR spectroscopic studies[9]. It has been established that the structured region of PrPc folds rapidly through a two-state mechanism without any intermediates, and without attendant aggregation, suggesting that PrPsc is not a conformational intermediate on the refolding pathway of the principal structured domain of the prion polypeptide[10]. PrPc has recently also been successfully converted into a fibrillogenic conformation *in vitro* through a chemical scheme involving refolding in the reduced state[11].

In recent years, a prion-like protein has been discovered in yeast[12]. The aggregated form of this protein is able to convert its normal (native) folded form into an aggregation-prone conformation upon contact[13], with the result that the aggregate-forming phenotype displays a cytoplasmic, rather than nuclear type of inheritance. This behaviour of the yeast prion lends critical support to the prion hypothesis, because with the mammalian prion itself it has proved to be impossible to demonstrate such conformational conversion through simple molecular contact *in vitro*. There are several questions relating to the prion hypothesis, therefore, that remain unanswered.

Summary of questions left unanswered

(i) PrPSc has not yet been demonstrated to induce misfolding of PrPc into PrPsc through ordinary contact *in vitro*, suggesting that there may be another protein (protein X) involved[1]. (ii) Further, no explanations have been found yet for how administration of PrPsc through the oral, or intraperitoneal, route(s) leads to neuroinvasive scrapie, as in kuru or new variant CJD[1]. (iii) It is also not known why prions sometimes occur in strains that cannot cross species barriers[14]. In this paper, it is mainly the second question that is addressed.

Mechanism of neuroinvasion: Current ideas

If peripheral administration of infectious prions causes neuroinvasive scrapie-like symptoms, something must physically carry the administered PrPsc to the brain or nervous system, or at the very least, carry a signal of some kind that is able to induce host prion misfolding into the PrPsc conformation. The cells of the lymphoreticular system would appear to be ideal candidates for such a task, since they (like certain other tissues, including nerve tissue) have long been known to express PrPc.

An apparently tenable hypothesis, therefore, would be that PrPsc encountered by lymphoreticular cells in a peripheral lymphoid organ (such as the mucosal-associated lymphoid tissue (MALT) underlying the epithelium of the gut) is able to induce a population of host PrPc molecules of lymphoreticular origin/affiliation into adopting the PrPsc conformation. Having thus 'taken over the baton', in a manner of speaking, lymphocytes carrying such freshly converted (host) PrPsc could then circulate through the body and induce PrPc of neuronal origin into adopting the PrPsc conformation, without the original infectious particle ever leaving the gut, or the peritoneum. Parts of this thesis have been tested by examining the effects of depletion/deletion and repopulation of B-cells in mice, and through knocking-out of the prion gene in mice used as B-cell donors, as described below.

B-cell depletion (support for current ideas)

Unlike healthy mice, which develop scrapie regardless of whether administration is done intracerebrally or intraperitoneally, SCID (severe combined immuno-deficient) mice lacking T-cells, as well as B-cells, have been found to develop scrapie only when PrPsc is administered intracerebrally[15]. Intraperitoneal adminstration does not lead to neuroinvasion. Significantly, repopulation of such SCID mice with B-cells derived from any healthy source re-establishes the transmissibility of scrapie from the peritoneum to the brain (however, repopulation by T-cells does not). Similarly, immunodeficient mice naturally lacking T-cells (but possessing B-cells) develop scrapie regardless of whether PrPsc is administered intracerebrally or intraperitoneally, whereas mice lacking B-cells (but possessing T-cells) do not develop scrapie when PrPsc is adminstered intraperitoneally. These observations clearly suggest a crucial role for B-cells in scrapie[15].

Prion gene knockout (problems with current ideas)

Disappointingly, the crucial role played by B-cells appears to have nothing to do with the expression of prions by such cells. In experiments reported very recently, it has been shown that repopulation of SCID as well as B-cell deficient mice by B-cells derived from knockout mice lacking the prion gene (and hence, the prion protein), re-establishes neuroinvasion in just as facile a manner as seen with B-cells bearing PrPc[16]. This has led to confusion about whether B-cells are central or peripheral players[1]. What role do B-cells have in neuroinvasion, if any?

An explanation already offered

One hypothesis, offered as an explanation, is that though B-cells lacking prion protein are genotypically prion-deficient, they can become phenotypically prion-bearing once they are in the body of the animal[1], through the 'jumping' of prion protein molecules from the surfaces of other cells on to the surfaces of B-cells, in a manner facilitated by cleavage and reattachment of the phosphatidylinositol glycolipid anchor holding PrP to a cell's surface. If indeed prion molecules can jump between cells in this manner, host PrP attached to lymphocytes might very well turn out to be involved in neuroinvasion.

This paper's perspective

I do not contest the explanation mentioned above (which, like any other hypothesis offered, can only be falsified by experiment; furthermore, nothing in the literature yet suggests that prions cannot jump between cells, so there is no reason to oppose the idea). However, if the assumption that prions can jump from other cells to knockout-derived B-cells turns out to be invalid, alternative scenarios will need to be considered. In the following sections, I develop a

hypothesis that offers one such alternative scenario. Let us begin by reviewing what is known about interactions between prions and the immune system to derive the alternative scenario.

The immune system and aggregates of misfolded proteins: self or non-self?

Attempts to generate antibodies against mouse PrPc, as well as against PrPsc, have shown that antibodies to either prion isoform can only be produced in knockout mice lacking the prion gene, or in a heterologous system (e.g. antibodies against mouse prion in hamsters, etc.), and not in normal mice[6]. The lack of generation of antibodies tends to be attributed to (a) recognition of both PrPc and PrPsc as 'self' molecules, by the immune system, and/or (b) the body's way of avoiding generation of antibodies to an entity that could naturally accumulate on the surfaces of lymphocytes, in the course of the disease.

Studies of the cross-reactivity of PrPc and PrPsc with panels of monoclonal antibodies generated separately against both prion isoforms in knockout mice, however, establish that while most antibodies from both panels cross-react with both PrPc and PrPsc, certain antibodies recognize only PrPc, or only PrPsc, but not both isoforms[17]. Such antibodies must specifically recognize conformational epitopes representing regions of the prion protein that differ in structure between the two isoforms (in fact, antibodies were generated and cross-reacted to establish and characterize conformational differences between PrPc and PrPsc).

This existence of such PrPsc-specific epitopes establishes that PrPsc is truly conformationally different from PrPc; not merely in a manner obvious to the spectroscopist, but also as perceived by the immune system of an animal that has never been exposed to the prion protein in any form. And yet, no antibodies are made. This raises an interesting question: Are misfolded (or alternatively folded) prion protein molecules 'self' or 'non-self' entities, from the point of view of the immune system?

Clonal deletion of cognate T-cells but not cognate B cells

Availability of cognate B-cells

If the misfolded form of a protein possesses one, or more, specific conformational epitopes that can give rise (in mice in which the gene encoding the protein has been knocked out) to monoclonal antibodies incapable of recognizing the native form of the protein, it may be argued that the misfolded form is 'non-self' as far as humoral (B-cell mediated) immunity is concerned, even in a normal mouse. Since naive B-cells bearing surface immunoglobulins (sIg) capable of binding to epitopes specific to the misfolded protein would ordinarily never have encountered such epitopes during initial development, there is no reason to believe that such B-cells would be clonally deleted in the bone marrow. (Only B-cells bearing immunoglobulins that recognize self epitopes are expected to be clonally deleted in the bone marrow.) Consequently, such B-cells may be expected to circulate through the peripheral lymphoid organs of all healthy individuals, every time a batch of haematopoietic cells combinatorially gives rise to diverse naive B-cells.

Non-availability of cognate helper T-cells

On the other hand, from the point of view of T-cell-mediated immunity, both the misfolded and native forms of any protein are clearly 'self' entities, since the covalent chemical nature of a misfolded protein (i.e. the amino acid sequence) remains unaltered by the act of misfolding. Peptides (T-cell epitopes) generated through proteolytic digestion of endocytosed (internalized) antigens, for display in complex with MHC molecules (on the surface of an antigen presenting B-cell) would, therefore, be the same for both the native and misfolded forms of any protein, assuming the misfolded form to be proteolytically susceptible (Figure 1).

T-cells bearing receptors that are cognizant of MHC-complexed peptides derived from such misfolded protein(s) could, therefore, be expected to be clonally deleted in the thymus during the early thymic education of developing T-cells. Consequently, such T-cells would not be expected to be found circulating in the healthy individual, ensuring that PrPsc-cognate B-cells would not get the chance to interact with such T-cells and give rise to a strong T-cell response.

No antibodies to (soluble) misfolded protein are expected

Given the manner in which B-cell activation usually occurs for soluble proteinaceous antigens, chances are low that antibodies would be produced against a misfolded protein if it were to remain soluble and resistant to aggregation. This is because naive B-cells bearing the requisite, cognate sIg receptors would first need to get activated (through binding, internalization, processing and presentation of the antigen), then proliferate, and finally mature into plasma cells capable of secreting antibodies; all three processes requiring the presence and cooperation of cytokine-secreting, helper T-cells bearing receptors capable of recognizing 'non-self' complexes of T-antigenic peptides and MHC molecules on the surface of the antigen presenting B-cell[18,19] (a mechanism commonly referred to as T-dependent (TD) activation). If B-cells binding these soluble, misfolded proteins were to receive appropriate cytokines nonspecifically from T-cells, or from other cells known to produce factors involved in B-cell differentiation, such as monocytes, macrophages, leukocytes, platelets and fibroblasts, a low-grade humoral response could potentially occur, but it is now well established that differentiation to immunoglobulin secretion is entirely dependent on T-cells, or their products[20–22].

No antibodies to insoluble aggregates are expected either

Even if the insoluble, aggregated form of a misfolded protein is endocytosed by a B-cell that bears cognate sIg molecules, and the endocytosed protein particle is processed into peptides that are then displayed with MHC molecules on the surface of the B-cell, no specific TD

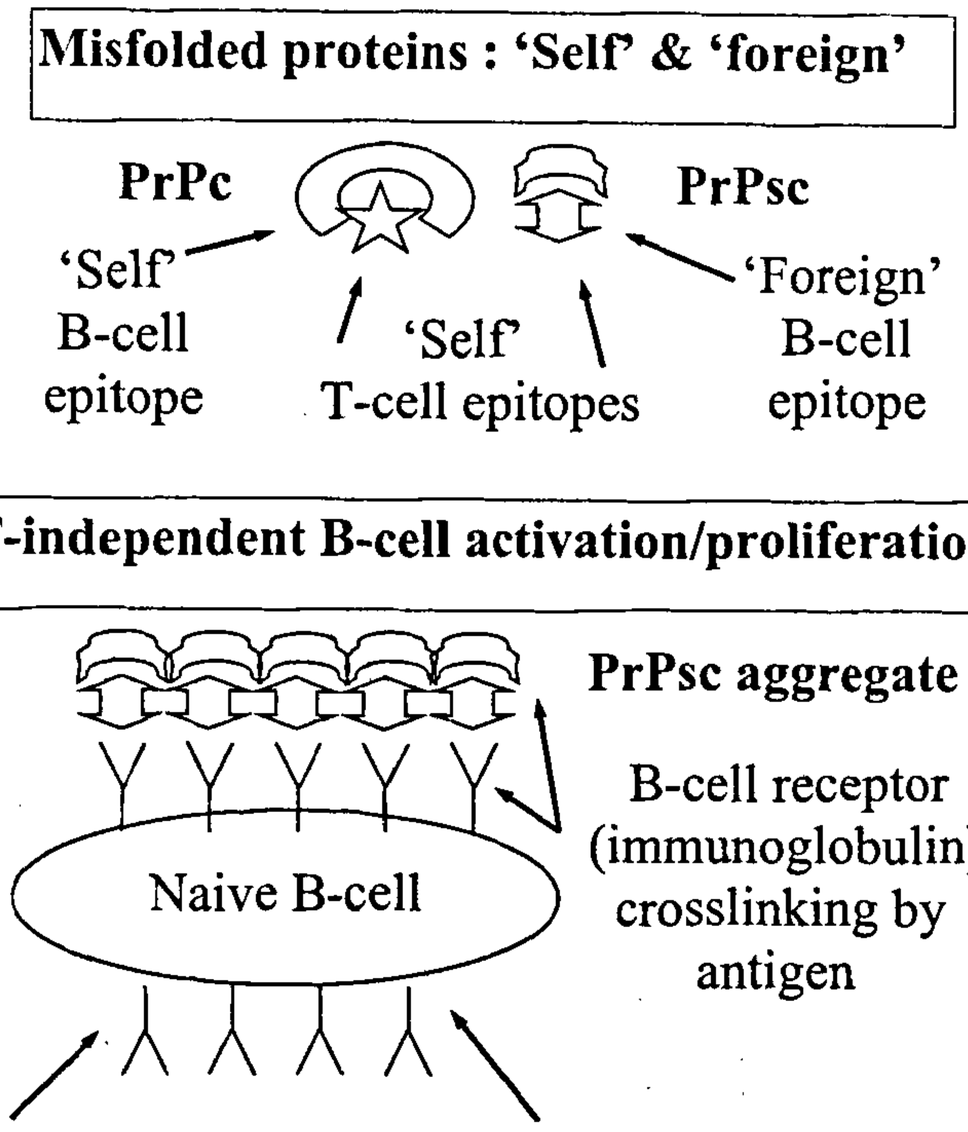

Figure 1. Schematic diagram of the key points of the hypothesis. The top panel illustrates how misfolded prion proteins must be 'foreign' where the B-cell system is concerned, and 'self' where the T-cell system is concerned. The middle panel shows how the aggregated form of misfolded prion protein (PrPsc) can activate naive B-cells carrying cognate immunoglobulin receptors, and cause such cells to proliferate throughout the body (simply by virtue of being able to crosslink B-cell receptors). The bottom panel shows a representative PrPsc epitope-specific immunoglobulin binding through induced-fit to a nascent prion polypeptide. Such binding is proposed to restrict the conformational freedom of the prion, and allow it to adopt only the PrPsc conformation.

activation can conceivably occur because there are likely to be no T-cells in circulation that bear receptors cognate to such peptide–MHC complexes (for reasons described previously, relating to clonal deletion). It may also be appropriate to point out here (though it is not really germane) that in the case of the prion rod, it is doubtful whether even proteolytic processing would occur if endocytosis by B-cells were assumed to occur, since prion rods have proved to be resistant to proteolysis *in vitro*[2]. Thus, both display of T-cell epitopes and recognition of such epitopes (if any happen to be displayed) by T-cells bearing appropriate receptors may be considered unlikely. As already pointed out, differentiation of B-cells into antibody-secreting plasma cells cannot occur without the involvement of T-cells or T-cell-derived cytokines[20–22]. Thus, any differentiation seen would only occur through the chance stimulation of relevant B-cells.

Thymus-independent (TI) activation of PrPsc-cognate naive B-cells

Importantly, if the misfolded form of a protein tends to aggregate, the requirement for T-cell involvement in simple B-cell activation and initial proliferation might not apply (though, as I emphasize throughout this paper, proliferation associated with differentiation cannot occur without T-cell-derived factors, naive B-cells are hyperresponsive to sIg crosslinking-mediated stimulation[23]). To appreciate this point better, let us remind ourselves that B-cells have an alternate mechanism of activation that is used for generating a humoral response to sugars and other substances that cannot be processed and displayed in complex with MHCs on the cell surface. This mechanism of activation (known as T-independent activation) involves the antigen-mediated cross-linking of sIg receptors on the surface of the B-cell[24] – done easily enough when the antigen is a large entity displaying the antigenic epitope repeatedly, in many copies. The binding of individual B-cell-borne sIg molecules to various, identical locations on the antigen(s) can remove the need for T-cell-based stimulation in B-cell activation and initial proliferation. Among the most well-cited examples of such responses are those to anti-IgM antibodies attached to a flat surface[25], in which case, there is neither endocytosis of the antigen, nor processing and display. In these and similar experiments, crosslinking of sIg of the IgM type present on the surfaces of B-cells, by the anti-IgM antibody on the flat surface (or by any other means of crosslinking of sIg) leads to cell-cycle entry and division of resting naive B-cells, and in certain cases production of interleukins by B-cells which can aid proliferation following activation. T-independent activation of such type does not lead to the production of memory B-cells. To elicit transformation of these activated and proliferated B-cells into antibody-secreting cells, cytokines are required to be delivered exogenously.

Aggregates like prion rods, which display (rather than bury) PrPsc-specific epitopes to the immune system (sufficiently well, in fact, to elicit antibody responses in knockout mice, as already discussed), would display such epitopes in many copies on their surfaces – simply by virtue of being composed of many molecules held together by non-covalent interactions, each bearing a copy of the epitope. Such aggre-

gates could thus be expected to bind B-cell-borne (sIg) immunoglobulins at many points, and thereby effect B-cell activation and initial proliferation (Figure 1).

Yet, no antibodies to mouse PrPsc are obtained in the mouse system[6], suggesting that further maturation of activated and tentatively proliferated B-cells, into memory cells and antibody-secreting plasma cells does not take place.

A note about non-production of antibodies

That no antibodies get made makes sense from common-sense and systems-engineering viewpoints. If misfolded and aggregated proteins in the vertebrate system were allowed to generate full-blown antibody responses, through a T-independent mechanism of activation, the body could well find itself fighting its own self a lot of the time. With over 50,000 proteins expressed in the 200-plus cell types making up the vertebrate animal, the probability of the odd protein getting misfolded and exposed to the humoral arm of the immune system, every now and then, is neither vanishingly small nor negligible. Given this, it would be wasteful for the immune system to have to generate antibodies to every 'self' protein happening to misfold quantitatively.

It is not inconceivable that the initial proliferation effected by the crosslinking of sIg leads eventually to apoptotic cell death, in the absence of appropriate stimulatory signals derived from T-cells. Indeed, it is well appreciated that sIg-mediated signalling can lead to cell activation, anergy, receptor editing or apoptosis, depending on cell type and developmental stage and also on the presence, or absence, of various cytokines and their receptors on the cell's surface[23]. Though absence of T-cells bearing cognate receptors would ensure that no strong immune response would occur (since sustained proliferation of fully differentiated plasma cells depends on T-cell-derived cytokines[22,23]), activation and proliferation, leading to possible accidental differentiation through non-specific contact with T-cells, could create problems for the immune system. Hence, the possibility appeals that an apoptotic mechanism exists to kill B-cells activated by misfolded self proteins. If misfolded prion aggregates remain bound to the cell surface, failing to get endocytosed and processed, this too may stimulate apoptotic mechanisms, but in the absence of information such speculation is rather premature.

Let us now apply the entire foregoing discussion to the examination of how neuroinvasion might be effected by prions?

Immunoglobulins as templates for prion misfolding: Induced fit binding and the determination of conformational fate

A nascent polypeptide chain folds into a structure dictated by its amino acid sequence. However, it can only be expected to do so, if it is allowed to do so, unhindered by interactions with other molecules. If something were to bind to a part of a nascent polypeptide chain in a conformationally-plastic stage of its folding, the consequences of such binding, in terms of the conformational fate of subsequent chain collapse and folding, could deviate significantly from the script.

Large molecules often bind to small, unstructured molecules (lacking adequate scope for autonomous adoption of unique structure on account of insufficient, intramolecular stabilizing interactions), through a mode of binding referred to as induced-fit (a term brought into vogue by Koshland and others; more information may be found in Creighton[26]). Induced-fit involves conformational adjustments in both binding partners. This mode of binding characterizes many molecular recognition events, from binding of hormones to their cognate receptors, to phage display-based screening of peptide libraries for ligand binding. If a large folded protein were to bind, through induced-fit, to regions of an incompletely-folded, or incompletely synthesized (i.e. nascent, or conformationally plastic) polypeptide while it was still attached to the ribosome, or in the process of being placed on the surface of a cell through translocation across the membrane, such binding could potentially restrict the conformational freedom of the nascent polypeptide, and force it to adopt structures that it would otherwise never have tended to adopt. In effect, what is likely to occur is the following scenario. A stretch of polypeptide that has not yet adopted a unique structure ends up populating a number of kinetically accessible conformations aided by the buffeting action of solvent molecules and the molecule's own thermal motions. One of these conformations happens to be recognized by a macromolecule which binds it tightly, thereby bleeding the equilibrium and causing the remaining population to repartition once again into all kinetically accessible states including the one recognized by the macromolecule present.

As outlined earlier, recently abandoned ideas about prion propagation invoked the transformation of host neuronal PrPc to PrPsc by B-cell-borne prion molecules[15,16]. These ideas assumed that molecules on the surface of a B-cell have access to neuronal prion molecules at a suitably (conformationally) plastic stage of folding. Without delving any deeper into that issue, and proceeding with the same assumptions regarding access of B-cells to host neuronal prion protein, I propose that we simply replace the B-cell-borne prions (now shown to be unnecessary) in the scheme, by B-cell-borne immunoglobulins capable of specifically binding to epitopes unique to PrPsc.

I further propose that we admit the possibility that immunoglobulins might induce the adoption of PrPsc-specific conformation(s) by epitope-defining regions in the prion polypeptide, in a manner characterized by (a) binding of the nascent prion molecule by the immunoglobulin, and (b) consequent narrowing down of the folding options of the prion molecule. In the event, such immunoglobulin-bound prions could be forced to fold into the scrapie conformation, at a site far from the original site of infection, initiating the propagation of scrapie in the central nervous system (Figure 1).

Antigen–antibody interactions are largely perceived to be rigid-body interactions, but recent studies indicate that peptides may bind to antibodies through induced-fit[27]. Then is the concept of chaperoning of protein conformation by antibodies/immunoglobulins, as proposed here, new? Somewhat anticlimac-

tically (from the point of view of novelty, and disappointingly, for the author), but extremely satisfyingly – from the point of view of the 'workability' of the present hypothesis, the answer turns out to be in the negative. Scanning of the biochemical and immunological literature in the PUBMED/MEDLINE database maintained at the National Library of Medicine, NIH, Bethesda, was performed, to search for reports published since 1969, discussing modulation of protein folding by immunoglobulins (using every conceivable combination of keywords). The search actually identified two papers from a group working at Tel-Aviv University, Israel[28,29]. These report that a monoclonal antibody raised against an epitope on carboxypeptidase A (which is distal to the enzyme's active site) significantly raises the yields of activity from refolding reactions of carboxypeptidase A, indicating that antibodies and surface immunoglobulins can bind to partially-structured states and influence the conformational fate of a protein molecule during structure formation. While these reports relate to antibody-based chaperoning to the native fold, the concept can be extended to chaperoning to a misfolded state, using cell-anchored immunoglobulins raised against epitopes specific to the misfolded state, as proposed in this paper.

What is of novelty here is thus the invocation of such immunoglobulin-mediated chaperoning in the formation of misfolded protein states and in the generation of disease, i.e. the idea that a disease-causing protein particle might use the body's immune system to trigger a chain of events leading to multiplication of an 'adaptor' molecule (also from the immune system), ultimately responsible for the multiplication of the disease-causing form of the protein.

Given the inability of PrPsc to cause conformational conversion of PrPc into PrPsc through ordinary molecular contact *in vitro*, there has been speculation and argument for several years now that a second molecule, referred to as protein X[30], probably helps induce cellular PrP to misfold in the image of PrPsc. Could PrPsc-specific sIg be protein X? This is an interesting question to think about. However, it must be borne in mind that there may be another molecule that functions as an adaptor within the nervous system, since intra-cranial injection of PrPsc leads to brain disease through propagation of the PrPsc conformation within the brain – obviously without any involvement of the immune system. Also, I would like to clarify that I am not proposing that every conversion of a prion polypeptide to the PrPsc conformation involves chaperoning by a PrPsc-specific sIg (if this were so, propagation of the disease would halt with the stopping of proliferation, or death, of cognate activated B-cells, and be limited by the total number of sIg molecules exposed to nascent prions in the nervous system); an unlikely situation for a disease that takes a long time to develop. While PrPsc appears to be unable to convert PrPc into PrPsc *in vitro*, it might cause misfolding of nascent (conformationally-plastic) prion polypeptide in the process of being translocated on to neuronal cell surfaces, such that sIg-based chaperoning leads eventually to self-chaperoning; the only limitation being that PrPsc can only convert nascent prions to the disease-causing conformation, and not already-folded PrPc. Why does the same not happen with B-cells? Maybe it is because naive B-cells are relatively short-lived, and because their cell-division and proliferation does not occur to a great extent in a location where they might be exposed to exogenous PrPsc (such as in the peritoneum, or the gut), whereas nerve cells tend to be very long-lived in the body (most nerve cells in the brain are created while in the womb, or as an infant) and probably, therefore, nerve cells keep recycling cell-surface proteins such as the prion protein, providing scope for at least some nascent molecules to be converted by the proliferated B-cell population.

Whatever the case, the hypothesis remains testable and falsifiable. I would propose the following tests: (1) B-cells extracted within days of peripheral administration of scrapie could be cultured in a dish and stimulated with all known cytokines to examine whether they can be stimulated to produce antibodies to PrPsc; (2) Such antibodies (or, for that matter, antibodies raised against PrPsc in knockout mice, or isolated and amplified *in vitro* using phage display antibody library-screening methods) could be reinjected into healthy animals to see if they develop scrapie. Alternatively, B-cells derived from prion-gene knockout animals that have, however, been exposed to scrapie, could be injected into healthy animals to see if the latter develop scrapie from prion-free B-cells.

The 'applied' angle (a postscript)

The above discussions suggest a possibly wider role for immunoglobulins in determining conformational fate. Certainly, immunoglobulins (and antibodies) raised against native epitopes of proteins of industrial, or therapeutic value could be put to use to coax recalcitrant proteins back to native state *in vitro*. However, such chaperoning could suffer from the antibody remaining bound to the folded protein. As an alternative, at least in some cases, antibodies raised against aggregated protein could aid folding to structural intermediate forms while at the same time blocking aggregation from occurring, for steric reasons (assuming that aggregates are frozen forms of structural intermediates; an idea for which experimental evidence is fast accumulating). Depending on the part of the molecule bound, if folding were to continue within molecules bound by such antibodies, affinity for the antibody could progressively reduce with progression of folding, giving rise ultimately to folded, antibody-free protein (an alternative scenario to what is proposed to happen here, with PrPsc).

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Design of a thermocycler based on light and air having optimal heat capacity

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This report describes the design of a 'fast' thermocycler for polymerase chain reactions (PCR). In this design of the thermocycler, the sample holders were placed over a copper foil made by electroforming, having an optimal heat capacity, and showing exact fitting of the sample holders. While an electric bulb was used as the source of heat, a muffin fan was used for cooling. A matched copper-wire resistance thermometer was used to monitor the temperature. Conventional poly-propylene tubes or ordinary glass capillaries were used as sample holders for DNA amplification. For exercising control over various timed events necessary for conducting PCR, the apparatus made use of the printer adapter of an IBM personal computer. The 'fast' thermocycler was evaluated for its response time, temperature profiles generated, and efficiency in amplifying DNA in the laboratory. Cost of the materials, excluding a PC, of this thermocycler works out around Rs 3000 only.

The polymerase chain reaction (PCR) technique has emerged as one of the most widely used techniques in molecular biology in the recent years[1]. The increase in the popularity of this technique has been accompanied by corresponding improvements in the apparatus used for performing it. A variety of engineering approaches to control temperature have been investigated for their applicability to the design of a PCR apparatus, including resistive heating and cooling by means of a refrigerant[2]; temperature cycling, using the Peltier effect[3], through the use of heated and chilled air-streams[4,5], and most recently, in a continuous flow manner[6].

In most of these designs the sample holder is placed inside a temperature-controlled metallic block, often weighing more than 500 g. Assuming that the specific heat of the material of metallic block is similar to that of aluminium, its heat capacity would be around 450 JK^{-1} which would be rather too high to heat or cool, for example, 10 samples, each of 50 µl, having heat capacity of 2.1 JK^{-1} only. The high heat capacity of the metallic block often limits the rate at which it could be heated or cooled. In thermocyclers based on heated and chilled air-streams, while the heat capacity of medium is quite small due to the low thermal conductivity of air, an air cyclone would be required to achieve uniformity of temperature.

In this report a thermocycler having a unique design of copper foil that holds sample holders having approximately 10 JK^{-1} heat capacity is described. In this design, copper foil is heated by placing it close to a halogen lamp, and subsequently cooling it by blowing a gentle stream of air over it. Owing to the optimal heat capacity of the copper foil, it can be heated and cooled quite rapidly. Furthermore, the high thermal conductivity of copper (unlike air) assures uniformity of temperature. A thermally matched temperature sensor is used to take the full advantage of fast temperature cycling of copper foil.

Features of the thermocycler

In this thermocycler samples can either be placed inside conventional poly-propylene tubes or else placed inside capillary tubes, and then placed on a blackened foil made by electroforming copper over poly-propylene tubes or capillary tubes. In the electroformed copper foil, tight fitting of the sample holder, and uniform and controlled thickness is ensured. A muffin fan blows air of ambient temperature over this copper foil. The temperature of the copper foil is maintained by controlling the light energy emitted by a 500 W halogen lamp. A 'matched' temperature transducer is used for monitoring temperature changes and communicating with the controller. The control is built around the parallel printer adapter of an IBM PC. Attaining of fast temperature cycling becomes possible with this thermocycler due to low heat capacity of the copper foil. For the incorporation of the above-outlined features in the design proposed here, the following rationale and overview of advantages were considered.

Optimal heat capacity of platform for sample holder

For the optimal heat capacity of the platform for sample holder, the heat capacity of such a platform should be nearly ten times the heat capacity of the sample holders to ensure heat transfer from the platform to the sample holders without any significant temperature change. A foil made by electroforming copper over poly-propylene tubes or capillary tubes served as a platform for sample holders. Thus by using an electroformed foil, the fitting of poly-propylene tubes or capillary tubes was observed to be exact, resulting thereby in maximum heat transfer. Furthermore, we observed that while a foil made by conventional machining process required highly specialized tools, the foil made by electroforming copper gave uniform deposition of copper with thickness (mass) that could be controlled.

Using light energy for heating

An electric bulb has a very fast response compared to nichrome wire heaters used in most designs. Faster ramp rate with practically zero 'dead time' was attained by heating the copper foil by the light of a 500 W halogen lamp. Phase control of AC mains was used to control the light intensity of the bulb, and thus the temperature of the sample holder.