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Properties of a disease-specific prion probe

Guy Gorochov
Immunologie A, Hôpital Pitié-Salpêtrière

Jean-Phillippe Deslys
Groupe d'Innovation Diagnostique et Thérapeutique sur les Infections à Prions

Eustache Paramithiotis
Caprion Pharmaceuticals

Marc Pinard
Caprion Pharmaceuticals

Trebor Lawton
INDEXX Laboratories

See next page for additional authors

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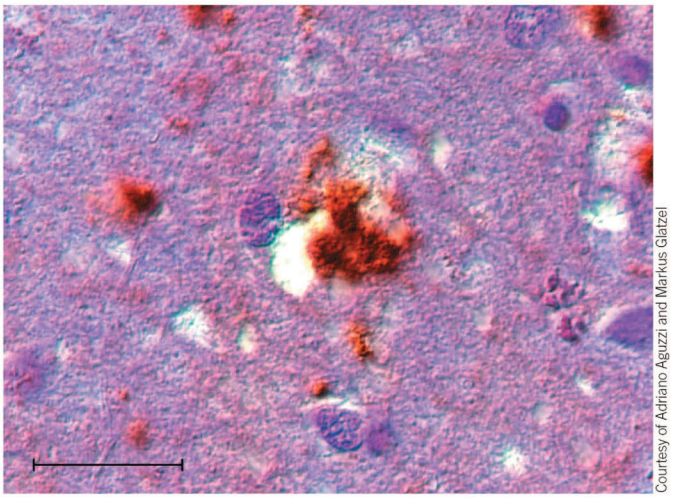
To the editor:

In a recently published article[1], Paramithiotis *et al.* describe antibodies specific for the prion Tyr-Tyr-Arg (YYR) repeat motif. These antibodies interact with the pathological isoform of the prion protein (PrP^{Sc}), but not with the normal cellular isoform (PrP^{C}). Because of this restricted specificity, they suggest that YYR-specific antibodies could be useful for the diagnosis and treatment of prion diseases (**Fig. 1**). The monoclonal antibodies, all of the IgM isotype, were produced by immunizing mice with a synthetic peptide (CYYRRYYRYY). When coupled to magnetic beads, these YYR-specific antibodies immunoprecipitate PrP^{Sc} much more efficiently than PrP^{C}. Notably, the Paramithiotis study did not rely on antibodies to YYR for specific detection of PrP. Their immunoblots were not ultimately probed with PrP^{Sc}-specific antibodies, but rather with 'regular' antibodies. The latter can detect PrP (but do not distinguish between PrP^{Sc} and PrP^{C}) in a precipitate that could include any protein containing solvent-accessible tyrosine and arginine residues.

This report is notably similar to that of Korth *et al.*[2], who described a Prp^{Sc}-specific IgM (designated 15B3) after immunizing with full-length recombinant bovine PrP. The 15B3 epitope consists of three separate, linear segments of PrP (15B3-1, 15B3-2 and 15B3-3). The YYR epitope (bold) identified by Paramithiotis *et al.* is included in or located near two of the 15B3 segments (underlined): GSDYEDR**YYR** (15B3-1) and **YYR**PVDQYS (15B3-2). Thus, these two independent studies relying on the same method of immunoprecipitation have identified similar IgM antibodies interacting with the same region on PrP, and possibly with the same YYR motifs.

The new reagents described could put an end to the quest for a PrP^{Sc}-specific antibody. Because the authors envision therapeutic use of the described antibodies, however, it seems appropriate to emphasize that YYR-specific antibodies could interact with any protein with tyrosine and arginine residues on its surface. In regard to PrP^{Sc} detection, it should be noted that no diagnostic application of 15B3 has been reported since the report of Korth *et al.* was published in in 1997. The availability of new diagnostic tests sensitive enough to ensure the protection of public health is an important issue[3]. For the design of such tests, there remains the choice between high-affinity antibodies that recognize both PrP^{Sc} and PrP^{C} but require prior elimination of PrP^{C}, and lower-affinity but PrP^{Sc}-specific antibodies.



Courtesy of Adriano Aguzzi and Markus Glatzel

Figure 1 Conversion of the normal form of the prion protein (PrP^{C}) to the pathogenic form (PrP^{Sc}) produces protein aggregates such as these. Scale bar, 20 μm.

COMPETING INTERESTS STATEMENT
The authors declare competing financial interests (see the *Nature Medicine* website for details).

Guy Gorochov[1] & Jean-Philippe Deslys[2]
[1]INSERM U543, Immunologie A, Hôpital Pitié-Salpêtrière, 75013 Paris, France.
[2]CEA/DSV/DRM, Groupe d'Innovation Diagnostique et Thérapeutique sur les Infections à Prions, CEA FAR, 18 Route du Panorama, BP6 92265 Fontenay-aux-Roses cedex, France.
e-mail: guy.gorochov@psl.ap-hop-paris.fr

1. Paramithiotis, E. *et al. Nat. Med.* **9**, 893–899 (2003).
2. Korth, C. *et al. Nature* **390**, 74–77 (1997).
3. Deslys, J.P. *et al. Nature* **409**, 476–478 (2001).

Paramithiotis *et al.* reply:

We did indeed use 'regular' PrP-specific antibodies for the immunoblotting and ELISA detection of PrP^{Sc} captured by our YYR-specific antibodies. However, we were also able to detect native PrP^{Sc} at the surface of scrapie-infected sheep dendritic cells using direct immunostaining protocols[1]. In addition, because YYR-specific antibodies do not react with brain homogenate by immunoblot, and do not seem to possess significant surface reactivity on normal dissociated neurons or lymphoid cells, this epitope is apparently rare in the structural proteome.

Gorochov and Deslys perceive a parallel between our hypothesis-driven discovery of the YYR epitope[1] and a previous report of a single putatively PrP^{Sc}-specific monoclonal antibody, 15B3 (ref. 2). We used biophysical methods to define the minimal peptide epitope YYR, then used YYR to immunize wild-type animals. This generated polyclonal antibodies and more than 40 PrP^{Sc}-selective monoclonal antibodies (including three IgGs). The 15B3 monoclonal antibody, in contrast, was raised in $Prnp^{-/-}$ mice immunized with recombinant bovine PrP. The 15B3 epitope was defined by reactivity to a 'gridded array' of bovine PrP 13-mer peptides staggered in register on a cellulose membrane[2]. Using this method, the 15B3

epitope was interpreted to be discontinuous, comprising three segments that occupy ~20% of the sequence of the PrP structured domain. If YYR were the 15B3 epitope, this sequence would have been centrally located in all three segments; however, only one segment contained the complete YYR motif at the extreme N terminus (segment II). In addition, the peptide-spotting method for epitope mapping revealed that several peptides recognized by 15B3 do not contain a YYR motif, whereas several peptides not recognized by 15B3 do contain YYR. The data, as presented[2], indicate that YYR is not the 15B3 epitope.

The usefulness of the YYR epitope in prion immunotherapy or immunoprophylaxis remains open until confirmed by experimental tests, as do its potential diagnostic applications.

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Eustache Paramithiotis[1], Marc Pinard[1], Trebor Lawton[2], Sylvie LaBoissiere[1], Valerie L Leathers[2], Wen-Quan Zou[3], Lisa A Estey[2], Julie Lamontagne[1], Marty T Lehto[3], Leslie H Kondejewski[1], Gregory P Francoeur[2], Maria Papadopoulos[1], Ashkan Haghighat[1], Stephen J Spatz[2], Mark Head[4], Robert Will[4], James Ironside[4], Katherine O'Rourke[5], Quentin Tonelli[2], Harry C Ledebur[1], Avi Chakrabartty[6] & Neil R Cashman[1,3,6,7]

[1]Caprion Pharmaceuticals, 7150 Alexander-Fleming, St-Laurent, Quebec H4S 2C8, Canada. [2]IDEXX Laboratories, 1 IDEXX Drive, Westbrook, Maine 04092, USA. [3]Centre for Research in Neurodegenerative Diseases, 6 Queen's Park Crescent West, University of Toronto, Toronto, Ontario M5S 3H2, Canada. [4]The National Creutzfeldt-Jakob Disease Surveillance Unit, Western General Hospital, Crewe Road, Edinburgh EH4 2XU, UK. [5]USDA-ARS-ADRU, 3003 ADBF, Washington State University, Pullman, Washington 99164-6630, USA. [6]Department of Medical Biophysics, University of Toronto, Ontario Cancer Institute, 610 University Avenue, Toronto, Ontario M5G 2M9, Canada. [7]Sunnybrook & Women's College Health Sciences Centre, University of Toronto, 2075 Bayview Avenue, Toronto, Ontario M4N 3M5, Canada.

1. Paramithiotis, E. *et al. Nat. Med.* **9**, 893–899 (2003).
2. Korth, C. *et al. Nature* **390**, 74–77 (1997).