

Properties of a disease-specific prion probe

To the editor:

In a recently published article¹, 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 (CYRRYYRYY). 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): GSDYEDRYYYR (15B3-1) and YYRPVDQYS (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 1997. The availability of new diagnostic tests sensitive enough to ensure the protection of public health is an important issue³. 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.

COMPETING INTERESTS STATEMENT

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

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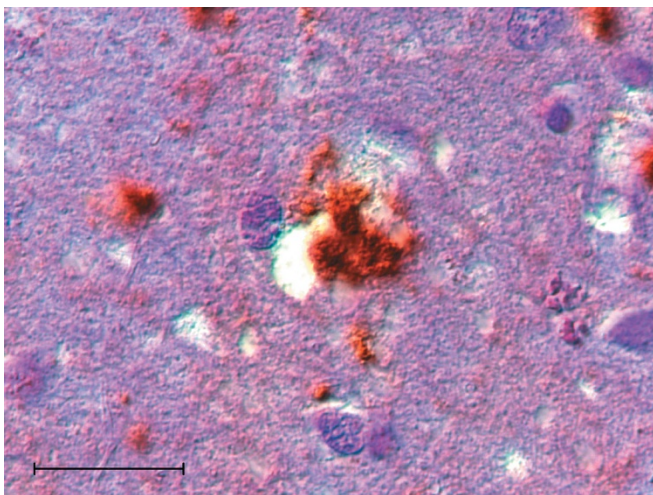
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1. Paramithiotis, E. *et al. Nat. Med.* **9**, 893–899 (2003).
2. Korth, C. *et al. Nature* **390**, 74–77 (1997).
3. Deslys, J.P. *et al. Nature* **409**, 476–478 (2001).

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 protocols¹. 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 epitope¹ 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 membrane². Using this method, the 15B3



Courtesy of Adriano Aguzzi and Markus Glätzel

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 μ m.