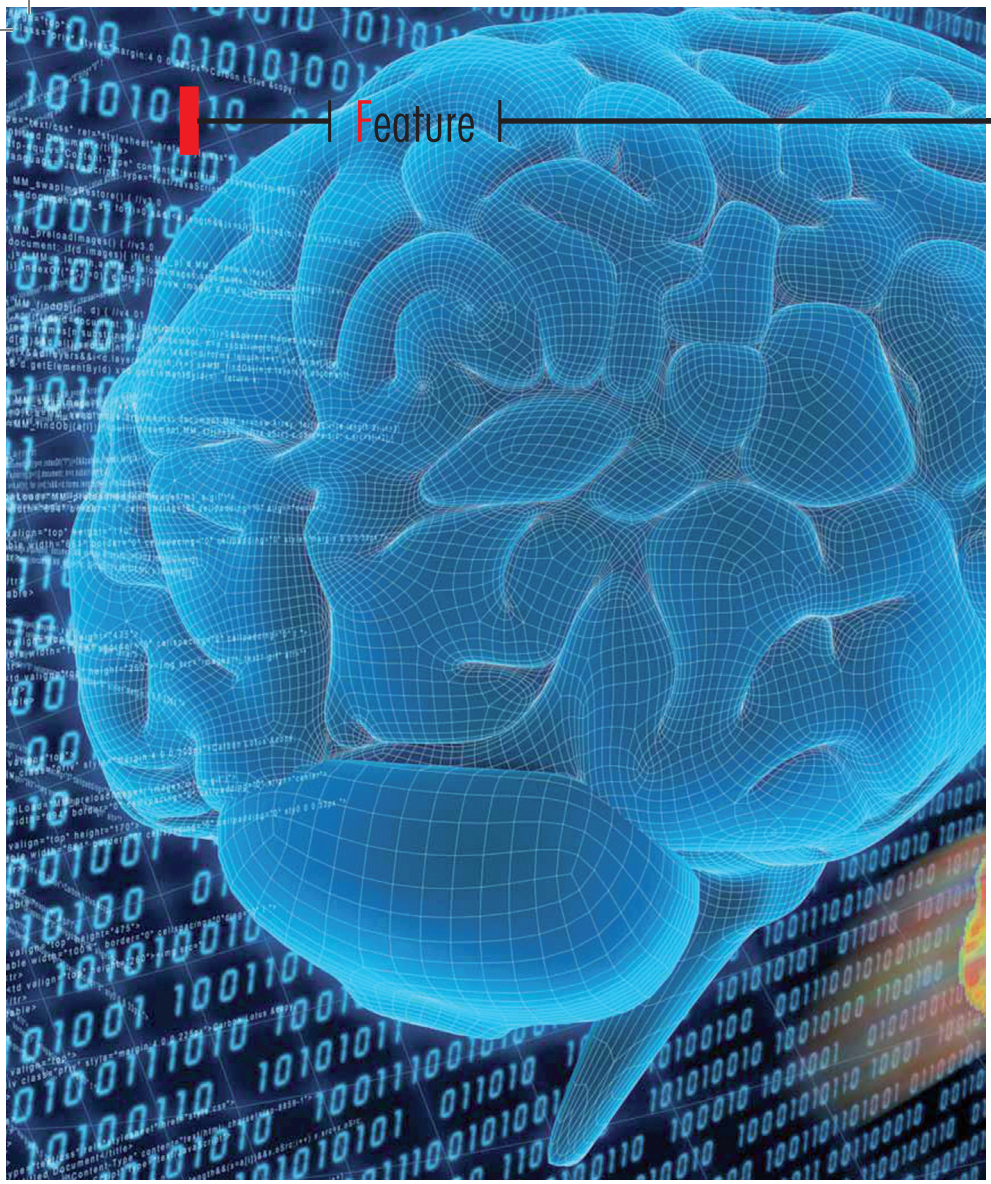

Combating Neurological Diseases with Antibodies

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The problem of neurological diseases is perhaps most urgent in India, where there is a particularly rapidly expanding overall and aging population, with 75 million over 60 years including 3.7 million with dementia. The complex and elusive nature of neurological diseases makes understanding mechanisms, diagnosis, treatment, management, and prevention overwhelmingly difficult. Reaching analogous success with the devastating neurodegenerative proteinopathies will continue to require scientists and clinicians alike to band together and sustain the momentum of the immunotherapy.

Ranging from the rare autosomal dominant monogenic inherited Huntington's disease (HD) to the multifactorial dementias, neurodegeneration is a pervasive problem worldwide. Collectively, neurological diseases are major causes of morbidity and mortality with devastating consequences for both patients and families. The problem is urgent, neurological sequelae are on the rise as populations age, and lifespan as well as environmental risk factors increase. Concomitantly, it is estimated that the prevalence of global dementia will increase from approximately 35 million in 2010 to about 120 million in 2050 (1).

India, already home to more than one out of 10 persons suffering from dementia, will grow from 1.15 to 1.53 billion between 2010 and 2030, surpassing China's population to become the largest country in the world. Hence, particular attention must be directed towards solutions in India and other rapidly expanding and developing countries where the impact of increasing neurological morbidity will be most felt. Stepping towards solutions with antibodies. Identifying and developing biomarkers is increasingly being recognised as the key for elucidating mechanisms of neurological disease (1). Likewise, in vitro studies, and animal models are also essential prerequisites for making the leap from the research laboratory to the patient bedside, a concept broadly known as translational medicine. The success of translational medicine relies on the collaboration, due diligence and creativity of both the research and medical communities. Antibodies are emerging as one of the most promising tools, as they are not only essential for basic biomedical research and biomarker discovery (Fig 1), but are also potential therapeutics.

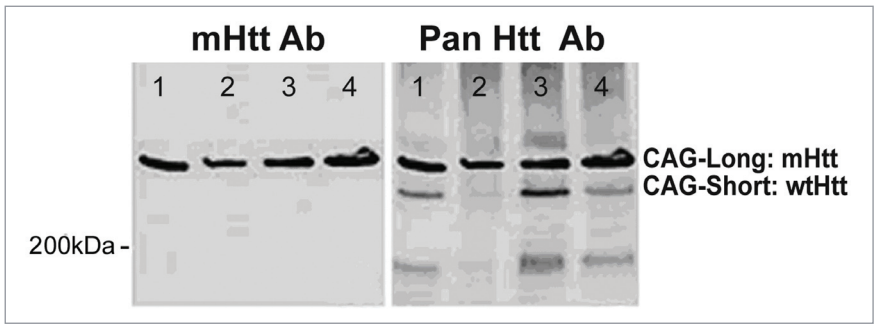

Fig-1. Monoclonal antibody clone 1A771 (IMGENEX) is specific for mHtt and an HD biomarker tool. Western blot analysis of Htt in lymphocytes from four HD patients, each expressing ~65 (mutant) and ~20 (normal) CAG repeats, with 1A771 mutant specific (A) or pan Htt (B) antibodies. Although both mHtt and wtHtt are expressed in the samples, 1A77A recognises only mHtt (mHtt is the biomarker signature of HD).

In the forefront are antibodies for combating proteinopathies, diseases that are riddled with the remarkable hallmark formation of misfolded protein aggregates. Neurodegenerative proteinopathies including Huntington's(HD), Parkinson's(PD) and Alzheimer's(AD) diseases as well as BSE (Mad-cow), and the TDP-43 diseases (ALS, various brain degeneration syndromes). Although proteinopathies have various genetic and multi-factorial origins, compelling evidence suggests that protein aggregation causes their respective disease pathologies. As such, an overarching vision and goal of translational medicine is that inhibition of protein aggregation would reverse or prevent the onset of symptoms.

On the horizon, one technology is intracellular antibody fragments (intra-antibodies) that disrupt intracellular protein aggregation by targeting specific parts or properties of a biomarker protein (3-5). For example, a given intra-antibody may target the disease-associated (mutant) protein itself, or an enzyme,

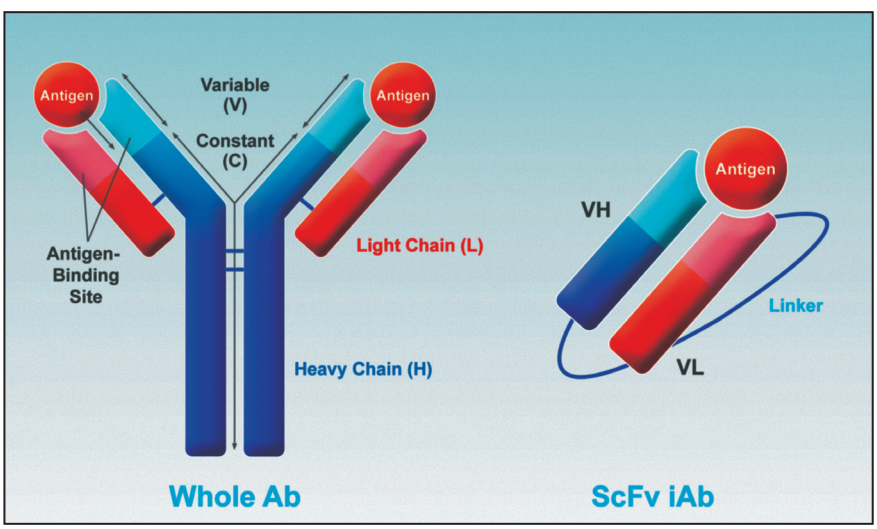

Fig-2. Comparison of whole antibody (Ab) and iAb derivative technology. The scFV format is a common type of iAb and contains only the VH and VL chains, held together by a linker peptide (3-5). cDNA from a monoclonal antibody hybridoma cell line can be used as starting material to generate an iAb of known specificity.

another protein or component of a protein complex required for the mutant protein to cause pathology.

Comparison of Intra Antibody to Whole Antibody Technology

Antibody technology is used to identify proteins or other chemicals (collectively called antigens) by immunodetection, and relies on specific interactions between antibodies and their antigens. The intra-antibody (iAb) or antibody fragment technology is based on derivatives of the standard whole antibody format (Fig 2). A whole IgG antibody like clone 1A771 (Fig 1) is a Y shaped molecule consisting of two heavy chains (~50 kDa each) and two light chains (~25 kDa each) linked (held together) by interchain disulphide bonds (Fig 2). Each heavy (H) and light (L) chain has a variable (V) and a constant (C) region. The whole antibody is not practical in the reducing environment inside the cell. In antibody fragment technology, the parts of the V regions that bind the antigen are leveraged to develop antibody fragments, which retain the binding specificity of whole antibodies. The single chain variable format (scFV), consisting of VH and VL segment held together by a linker peptide is a widely used design for iAbs. Examples of iAbs directed against HD, PD, and AD are shown in Table I.

Rationale for Targeting Neurodegenerative Proteinopathies with iAbs

The development of iAbs that disrupt or prevent protein aggregation has a tremendous potential for a pipeline of macromolecular drugs for treating proteinopathies. Considerable intra-antibody technology is being directed towards HD (mHtt aggregation), AD (beta amyloid plaques), and PD (Lewy bodies containing alpha-synuclein aggregates) for a number of reasons (2-5). Firstly, AD and PD are the first and second most prevalent neurodegenerative diseases, respectively; more than 1,000 patients are diagnosed with AD daily and about two per cent of people over 65 years suffer from PD. HD, albeit rare with a worldwide prevalence of about 0.01 per cent, is particularly ominous as each child of a patient has a 50 per cent risk of developing the disease. These children must wait in horror for impending disease symptoms that typically appear in midlife.

Detection of Neurological Disease by Antibodies

AD and PD both have multifactorial causes resulting from the interplay of genetic and environmental risk factors, whereas HD clearly results from the inheritance of a huntingtin (Htt) gene, which has an expansion of the normally occurring glutamine repeat (CAG) in exon 1. The monogenic pathology of HD serves a useful model for explaining integral pathology of a proteinopathy. When the Htt CAG expansion is greater than 36, the Htt protein misfolds into an abnormal conformation and accumulates as intracellular aggregates. Both iAbs and whole antibodies can be designed that specifically recognise abnormal, but not normal, protein conformations like iAbs in Table I and antibody clone 1A771 for HD (Figure 1). As HD is a dominant gene mutation, patients

Disease Target	iAbs	Effects
AD	hk14	Cleaves ABeta42
AD	C23.5	Mimics alpha-secretase cleavage
AD	sFvBeta1	Reduces ABeta production and toxicity in cell culture
AD	H1v2	Reduces ABeta aggregation in vitro
AD	svFv9, scFv40.1, svFv42.2	All reduce ABeta plaques in mice
HD	C4	Reduces mHtt aggregation and increases survival in drosophila
HD	VL12.3, 6E	Both reduces mHtt aggregation and neuronal death in mice
HD	Happ1	Reduces mHtt aggregation and toxicity in cell culture and organotypic brain slices, at least five different mouse models support therapeutic utility
HD, PD	D5	Reduces mHtt aggregation, but increases toxicity in HD cell culture. Reduces alpha synuclein aggregation and toxicity in PD cell culture. Raised against mutant alpha synuclein. Recognises a conformational epitope common to mHtt and mutant alpha synuclein that is not dependent on amino acid sequence, per se
PD	NAC32, D10	Reduce alpha synuclein aggregation and toxicity in cell culture

Table I. AD, HD, and PD iAbs are at various stages of development in the quest to move the immunotherapy from research to humans. Cell-free, in vitro cell culture, in vitro organ and in vivo drosophila and animal model system examples are shown (3).

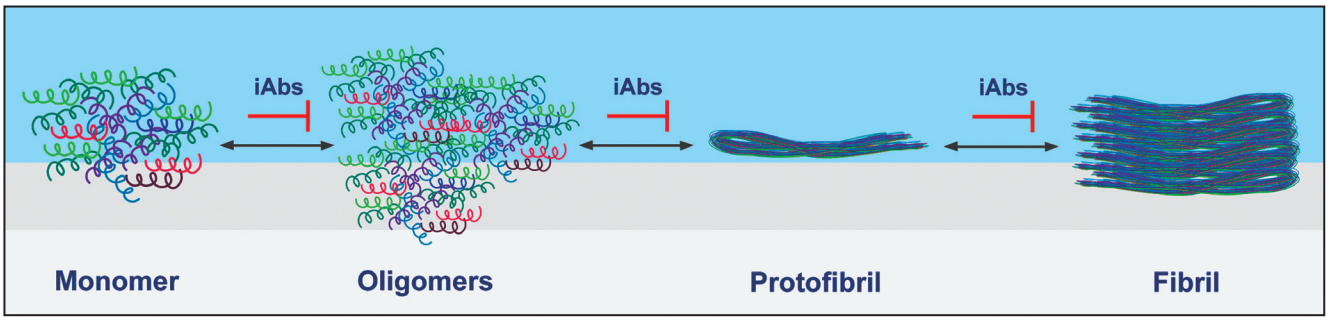

Fig-3. The misfolded, aggregating proteins of neurodegenerationproteinopathies assume multiple, common abnormal conformations. The goal of iAbs translational medicine is to block abnormal conformations and restore normal protein function.

will express both normal, wild-typeHtt (wHtt) and mutant Htt (mHtt) as seen in Figure 1. However, mHtt can co-aggregate with normal mHtt inhibiting its function, and mHtt aggregates also sequester other proteins including transcription factors, vesicle-associated proteins hampering their function.

These toxic effects lead to cortical atrophy and progressive motor and cognitive decline, eventually resulting in death. Historically, treatments have been aimed at symptom management rather than modification of the underlying mHtt disease pathology. It is important to recognise that the spectrum of neurological disease is large, encompassesing a myriad of underlying mechanisms.

Fortunately, the increasing availability of research antibodies to proteins involved in both normal and aberrant signaling pathways are helping to elucidate biomarkers and develop potential diagnostics for a broad spectrum of conditions.

For example, there are antibodies recognising active caspases found in dying neurons, Parkin (PD signaling), prions (Mad-cow), DARPP-32 (master brain signaling regulator), and cytochrome c (released into the cytoplasm during cell death) to name a few.

Conclusion and Future Directions:

Although they differ in function, sequence and size, the proteins that cause HD, AD, and PD assume similar structures including monomers, small aggregated oligomers, Beta sheet organised protofibrils, and large aggregated cross-B-sheet amyloid fibrils (Fig 3) (3). As detailed in Table I, emerging evidence suggests that iAbs specifically targeting conformation-specific or eptiope-specific regions of proteins causing these diseases can inhibit both aggregation and toxicity.

Although iAbs show great promise, much more needs to be done to move from cell-free, in vitro cell and in vivo mouse models to human therapies. Nonetheless, as Southwell and Patterson (3) so eloquently stated "one should recall the many missteps and the fine-tuning that was required to achieve the nearly uniform success we now enjoy with bone marrow, kidney, and heart transplant procedures."

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