Institution: University of Oxford

Unit of Assessment: 4

Title of case study:

Identifying Autoimmune Diseases of the Nervous System and Improved Methods to Diagnose Them

1. Summary of the impact

Autoimmune diseases are caused when the body attacks itself by making auto-antibodies against its own proteins. Research by Angela Vincent and her colleagues in Oxford over the past 20 years has led to the identification of specific auto-antibodies that cause several unexplained (and sometimes fatal) neurological diseases. The auto-antibodies are now used to diagnose these diseases, enabling appropriate treatment. Vincent's research has also developed better and more sensitive methods for detecting auto-antibodies. These methods are now widely used, both in her own lab (where she provides a clinical service, conducting over 100,000 tests since 2008) and worldwide. The tests have also been patented and licensed, generating over £500,000 since 2008.

2. Underpinning research

Identifying antibodies that cause brain diseases

The immune system makes antibodies to help us fight infection, but we can also make antibodies against our own proteins ('auto-antibodies'), causing so-called autoimmune diseases. The diagnosis is made by detecting auto-antibodies in blood samples. Once diagnosed, these diseases improve substantially when antibody levels are reduced by immunosuppression (e.g. by steroids or plasma exchange); therefore effective diagnosis is imperative for effective treatment.

Some autoimmune diseases affect the nervous system, such as myasthenia gravis, in which the connections between nerves and muscles are impaired. This is usually caused by antibodies targeting the acetylcholine receptor, but about 15% of cases do not have this antibody. In 2001, Vincent's group showed that many of these unexplained cases are caused by auto-antibodies against a different protein, called MuSK (Hoch et al., 2001).

Subsequently, Vincent showed that auto-antibodies not only cause diseases of nerve and muscle, but also of the brain itself, including forms of encephalitis (brain inflammation). As the cause of these diseases was previously unknown, and wrongly attributed to a viral infection, this discovery has had a major impact on the field. The following two examples illustrate the key research:

i. Discovering antibodies against potassium channel complex proteins ('VGKC') that cause a distinct form of encephalitis (Irani et al., 2010a) often preceded by a unique form of epilepsy (Irani et al., 2011); over 100 cases are now diagnosed in the UK each year.
ii. Showing that antibodies against the NMDA receptor cause a separate and severe form of encephalitis. Although also reported by an American group, Vincent's work emphasises that this is common, occurring in patients of all ages, and often without an underlying tumour (in contrast to what had been believed previously; Irani et al., 2010b). Unexpectedly, Vincent's recent work indicates that these antibodies may also cause a schizophrenia-like illness; this finding has major implications for its diagnosis and treatment, and is being followed up in prospective studies in the UK and internationally.

Crucially, autoimmune brain disorders often respond dramatically to immunosuppressive therapy (e.g. Irani et al., 2010a, 2011). Thus, Vincent's research has also had a major impact on treatment and outcome. And, the recognition that antibodies cause brain disease, even when not associated with tumours, has changed perceptions such that "autoimmune" is now high on the list of differential diagnoses, allowing earlier diagnosis and treatment. E.g.: *'…patients with antibody-associated encephalitis in otherwise unexplained cases…highlights the epidemiological importance of these antibodies … Prompt distinction between causes of acute encephalitis is essential to direct appropriate management…*' (Granerod et al, Lancet Infectious Diseases 2010; 10: 835-44.)

As well as identifying new auto-antibodies and the clinical syndromes they cause, Vincent's research has helped reveal the mechanisms by which the antibodies lead to disease, using a

range of cellular and animal models. She has also clarified the specific molecular target of the antibodies, e.g. the individual proteins within the VGKC complex (Irani et al., 2010a; Section 4).

Developing better methods to detect auto-antibodies

A parallel strand of research carried out by Vincent and colleagues has been to develop more sensitive methods ('assays') to detect the auto-antibodies, and thus aid diagnosis (Leite et al., 2008; Waters et al., 2008). The latter paper concerns aquaporin 4 (AQP4) antibodies, which cause a disease called neuromyelitis optica; the article shows that the new assay for the antibodies from Vincent and colleagues, outperforms the standard method. This superiority was confirmed in an international study (Waters et al., Neurology 2012;78:665-71) and independently by a Japanese group (Sato et al., Neurology 2013;80:2210-6).

3. References to the research

Hoch W, McConville J, Helms S, Newsom-Davis J, Melms A, **Vincent A** (2001) Auto-antibodies to the receptor tyrosine kinas MuSK in patients with myasthenia gravis without acetylcholine receptor antibodies. Nature Medicine 2001; 7: 365-368. DOI: 10.1038/85520. ***Identifies MuSK antibodies as a distinct cause of myasthenia gravis.*** Cited 393 times.

Irani SR, Alexander S, Waters P, Kleopa K, Pettingill P, Zuliani L, Peles E, Buckley C, Lang B, **Vincent A** (2010a) Antibodies to Kv1 potassium channel-complex proteins leucine-rich, glioma inactivated 1 protein and contactin-associated protein-2 in limbic encephalitis, Morvan's syndrome and acquired neuromyotonia. Brain 133:2734-48. DOI: 10.1093/brain/awq213. ***Identifies the specific targets of anti-VGKC antibodies. Also describes the clinical features and treatment responses. The work led to the patent and license described below.*** Cited 169 times.

Irani SR, Bera K, Waters P, Zuliani L, Maxwell S, Zandi M, Friese M, Galea I, Kullmann DM, Beeson D, Lang B, Bien C, **Vincent A** (2010b). N-methyl-d-aspartate antibody encephalitis: temporal progression of clinical and paraclinical observations in a predominantly non-paraneo-plastic disorder of both sexes. Brain 133:1655-1667. DOI: 10.1093/brain/awq113. Cited 159 times.

Irani SR, Michell AW, Lang B, Pettingill P, Waters P, Johnson MR, Schott JM, Armstrong RJE, Zagami AI, Bleasel A, Somerville ER, Smith SM, **Vincent A** (2011) Faciobrachial dystonic seizures precede Lgi1 antibody limbic encephalitis. Ann Neurol 2011; 69:892-900. DOI: 10.1002/ana.22307. ***A study reporting 29 patients with a newly identified form of epilepsy, unresponsive to standard drugs but responding dramatically to immunotherapy. A collaboration with the Institute of Neurology and others.*** Cited 70 times.

Leite MI, Jacob S, Viegas S, Cossins J, Clover L, Morgan B, Beeson D, Willcox N, **Vincent A** (2008) IgG1 antibodies to acetylcholine receptors in 'seronegative' myasthenia gravis. Brain 131: 1940-52. DOI: 10.1093/brain/awn092. ***Demonstrates that some acetylcholine receptor and MuSK antibodies are not detectable by the standard 'radioimmunoprecipitation' assays used previously, but are positive when exposed to cells expressing the native antigens on the cell surface (a 'cell-based assay').*** Cited 115 times.

Waters P, Jarius S, Littleton E, Leite M, Jacob S, Gray B, Gerlades R, Vale T, Palace J, Maxwell S, Beeson D, **Vincent A** (2008) Aquaporin-4 antibodies in neuromyelitis optica and longitudinally extensive transverse myelitis. Arch Neurol 65:913-919. DOI: 10.1001/archneur.65.7.913. ***A new cell-based AQP4 assay, shown to be superior to the previous method.*** Cited 143 times.

Grant support

The research was supported over 20 years by MRC via Programme Grants to Newsom-Davis, Vincent and Willcox, and over the last 7 years via project grants, and by training awards to clinicians supervised by Vincent (e.g. Camilla Buckley, Sarosh Irani). The recent grants include:

- DANA Neuroimmunology award to Vincent, 2004-7. ~£150K.
- MRC Grant to Buckley, Fugger and Vincent, 2005-7. ~£200K
- NIHR Clinician Training Fellowship to Dr S Irani; supervisor Vincent, 2007-2010. £240K.

Vincent has been supported by HEFCE since 1992. She retired formally in 2008 but has continued

to be employed by the University of Oxford with a 2 day per week (0.4FTE) contract from 2012-17.

4. Details of the impact

Impacts on clinical practice: diagnosis and treatment

MuSK and myasthenia gravis. Vincent's work has had an impact on the diagnosis and treatment of this condition. European Federation of Neurological Societies guidelines for myasthenia gravis (Section 5, Source 1) highlight that the MuSK antibodies have therapeutic relevance. The two standard treatments for myasthenia gravis are anticholinesterase drugs, and removal of the thymus gland ('thymectomy'). In MuSK-positive cases, the guidelines state that thymectomy '*should not be recommended',* and anticholinesterases used '*with some caution*'. These treatment implications were not present in the previous (2006) version of the guideline.

Autoimmune encephalitis. Guidelines for encephalitis now recommend testing for auto-antibodies (e.g. NMDA receptor, VKGC): Association of British Neurologists and British Infection Association National Guidelines and European Federation of Neurological Societies (2011):

- The British guidelines for adults (Section 5, Source 2) includes the statements: "*Metabolic, toxic, autoimmune and non-CNS sources of sepsis as causes for encephalopathy should be considered early in patients…(B, III)." "The differential diagnosis of acute encephalitis is broad, encompassing infectious, para-infectious immune-mediated, autoimmune, metabolic, vascular, neoplastic, paraneoplastic, and toxic aetiologies as well as brain dysfunction due to systemic sepsis (Tables 2, 3 and 9)".* 5 (of the 7) papers cited as evidence guiding their recommendations about autoimmune encephalitis are from the Vincent group.

- The British guidelines for children (Section 5, Source 3) include the statement: "*Patients presenting with a sub-acute (weeks to months) encephalitis should trigger a search for autoimmune, paraneoplastic, metabolic aetiologies (C, III*)." The review cites a Vincent group paper (Irani et al., 2010b) as evidence for NMDA receptor antibody encephalitis.

Vincent's research has also had a dramatic impact on how patients with encephalitis are managed, since a positive antibody test leads directly, in most cases, to use of immunosuppressant treatments. This would not have occurred otherwise. Data from UK and European clinicians indicate clearly the improvement in outcomes following immunotherapy (e.g. see Irani et al., papers cited in Section 3, plus the review by Granerod et al, Lancet Infectious Diseases 2010;10:835-44).

Providing a clinical diagnostic service

Vincent's group provides a clinical service, jointly with the local NHS Immunology lab, testing for all the antibodies described above, and others (Section 5, Source 7). This was the first such service in the UK, and remains by far the largest both for testing NHS samples and for providing clinical advice. The number of requests, and positive results, has risen dramatically, with over five times as many tests performed in 2012 as in 2007 for the antibodies mentioned in this case study (Table). For example, in 2007 there were 605 requests for AQP4 antibodies and 85 positive results; in 2012 the corresponding figures were 4867 and 356. About 80% of requests are from NHS clinicians, the rest mostly from Europe. The ~£100K annual surplus made by the testing service is used to support Vincent's research.

Year	Number of tests for the antibodies	Number of positive results
2007	5198	291
2008	9784	470
2009	13088	761
2010	18303	1098
2011	23015	1510
2012	27177	1702
2013, to July	15460	728

Impact of improved methods to detect auto-antibodies

The live cell-based assays developed by Vincent, e.g. for AQP4, have now been adopted by other centres (Source 5), notably the Mayo Clinic, USA, which tested 20,334 patients in 2011. Also, Vincent has provided resources and guidance to help set up the assays for centres in France, Greece, Hungary, Australia, Korea and China. Her work also led to establishment of an NHS clinical service, jointly with the University of Liverpool, for patients with AQP4 disease, with greatly improved management of this life-threatening disease.

The greater sensitivity of Vincent's AQP4 method was confirmed by Iorio et al., (Section 5, Source 6) who state: '*These observations predict greater disease sensitivity and specificity for the cell-based serological assays'.* This newAQP4 detection method has also been applied in a Japanese study to show that AQP4 antibodies contribute to a wider range of diseases than had been suspected, extending the clinical impact (Sato et al., Neurology 2013; 80: 2210-6).

Commercial impacts: Patents and licensing

MuSK antibody for diagnosis of myasthenia. With Dr W Hoch, University of Tuebingen) **PCT/GB01/02661.** Filed June 16th, 2001, subsequently filed in Europe and Japan. Licensed to Athena Diagnostics USA who sublicensed to RSR Ltd in 2003; income to Oxford University ~£1M, of which £500K has been received since 2008. (Section 5, Source 8).

Autoimmune Disorders (VGKC-complex proteins, LGI1, CASPR2, contactin-2) **PCT/GB2009/051441.** Filed 26.10.2009. Licensed to Euroimmun AG, Luebeck, for the development of diagnostic tests. Income to Oxford University in 2013: £65K. **(**Section 5, Source 9).

New method (Membrane vesicles for antibody assays) **Isis project 9557 (N118855-GB),** patent application number 1310855.0, filed 18 June 2013.

Other impact on patients/carers

Public information about encephalitis now includes description of autoimmune encephalitis, reflecting the identification of this category of disease from the work of Vincent, and others (Section 5, Source 10).

5. Sources to corroborate the impact

Clinical practice guidelines for myasthenia gravis and the relevance of the MuSK subtype:

1. Skeie GO, et al., Guidelines for treatment of autoimmune neuromuscular transmission disorders. Eur. J. Neurol. 2010; 17: 893-902.

Clinical practice guidelines describing importance of antibody testing in encephalitis:

2. Solomon T et al., on behalf of the National Encephalitis Guidelines Development and Stakeholder Groups. Management of suspected viral encephalitis in adults. Association of British Neurologists and British Infection Association Guidelines. J. Infect. 2012 64:347-73.
3. Kneen R et al., on behalf of the National Encephalitis Guidelines Development and Stakeholder Groups. Management of suspected viral encephalitis in children. Association of British Neurologists and British Paediatric Allergy, Immunology and Infection Group national guidelines. J. Infect. 2012 64:449-77.
4. European Federation of Neurological Societies 2011 guidance. Chapter 6: Use of antibody testing in nervous system disorders. DOI: 10.1002/9781444328394.ch6.

Evidence of other clinical impacts:

5. Letter on file from Prof M. Dalakas, Thomas Jefferson University, Philadelphia, and University of Athens, confirming the global impact of Vincent's discoveries and of her testing service.
6. Iorio et al, Astrocytic autoantibody of neuromyelitis optica (NMO-IgG) binds to aquaporin-4 extracellular loops, monomers, tetramers and high order arrays. J. Autoimmun. 2013; **40** 21-27.
7. Description of Vincent's NHS diagnostic testing service: http://www.oxfordlaboratorymedicine.co.uk/laboratory-services/immunology/neuroimmunology/

Evidence of commercial impacts:

8. Letter on file: Athena Diagnostics, USA regarding MuSK license and other contributions.
9. Letter on file: Head of Euroimmun AG, Germany, regarding several antibody licenses.

Public education:

10. Website describing autoimmune encephalitis and importance of NMDA receptor antibodies: http://www.encephalitis.info/information/types-of-encephalitis/