strong regulatory components thereafter. Recovery of local complement is quite rapid as there is a local complement synthesis. Besides the abovementioned anti-inflammatory effect (1) immunoglobulins through the V-connected network have in addition an immediate and direct influence on biding of pathological autoantibodies, as shown here and elsewhere; (2) interacting with T- and B-cell receptors, they can influence long-term the immunoglobulin repertoire and (3) they can influence dendritic cell maturation.[25] In GBS, an acute disease not being in a steady-state, the mild regulatory effects of plasmaphaeresis apparently are sufficient to mediate an improvement. In MMN, however, a chronic condition having established some level of steady state, the more powerful immunomodulatory effect of IVIG is needed to achieve an improvement.

A better understanding of the IVIG action mechanism will aid in developing more effective treatments for MMN. Moreover, more rational treatment may make use of complement inhibitors. The murine model of Fisher syndrome has been treated successfully with eculizumab, a humanised monoclonal antibody that binds to and blocks cleavage of C5.[26] Several serine proteases activate the classical and alternative complement system pathways, and a synthetic serine protease inhibitor, nafamostat mesilate, inhibits complement deposition and prevents sodium channel cluster disruption in the AMAN rabbit model.[27] In our in vitro study, nafamostat mesilate blocked the C3b deposition mediated by anti-GM1 IgM antibodies in MMN (data not shown). Complement inhibitors, therefore, may provide optional treatment of MMN.

In conclusion, our findings proved that there is a binding of pathogenic anti-GM1 autoantibodies from MMN sera to the target antigen GM1 in vitro. This suggests a similar effect happening during MMN progression. As a consequence of formation of antigen-antibody complexes, local complement activation occurs from C1q to C5b-9, the latter representing the tissue-damaging membrane attack complex. The results presented support the view of multi-site concomitant IVIG action benefitting MMN patients. They indicate that IVIG counteracts the immune complex-initiated, complement-mediated tissue damage activation at various stages; by anti-idiotypic activity, as well as inhibition of complement deposition by scavenging nascent C3b and, probably more important, by specific inhibition of the alternative pathway of C3 convertase assembly.[23 24] As a consequence of reduced C3 activation, generation of the membrane attack complex and tissue damage are reduced. In other words, IVIG may inhibit complement activation and in situ deposition, thereby leading to improvement of muscle strength.

Competing interests None.

Ethics approval This study was conducted with the approval of the Dokkyo Medical University, Tochigi, Japan.

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES

1. **Nobile-Orazio E.** Multifocal motor neuropathy. *J Neuroimmunol* 2001;**115**:4–18.
2. **Susuki K,** Rasband MN, Tohyama K, *et al*. Anti-GM1 antibodies cause complement-mediated disruption of sodium channel clusters in peripheral motor nerve fibers. *J Neurosci* 2007;**27**:3956–67.
3. **Willison HJ,** Halstead SK, Beveridge E, *et al*. The role of complement and complement regulators in mediating motor nerve terminal injury in murine models of Guillain–Barré syndrome. *J Neuroimmunol* 2008;**201-202**:172–82.
4. **van Schaik IN,** Bouche P, Illa I, *et al*. European Federation of Neurological Societies/Peripheral Nerve Society guideline on management of multifocal motor neuropathy. *Eur J Neurol* 2006;**13**:802–8.
5. **Yuki N,** Tagawa Y, Irie F, *et al*. Close association of Guillain–Barré syndrome with antibodies to minor monosialogangliosides GM1b and GM1α. *J Neuroimmunol* 1997;**74**:30–4.
6. **Hirabayashi Y,** Nakao T, Matsumoto M, *et al*. Improved method for large-scale purification of brain gangliosides by Q-sepharose column chromatography: immunochemical detection of C-series polysialogangliosides in adult bovine brains. *J Chromatogr* 1988;**445**:377–84.
7. **Sekul EA,** Cupler EJ, Dalakas MC. Aseptic meningitis associated with high-dose intravenous immunoglobulin therapy: frequency and risk factors. *Ann Intern Med* 1994;**121**:259–62.
8. **Uetz-von Allmen E,** Sturzenegger M, Rieben R, *et al*. Antiganglioside GM1 antibodies and their complement activating capacity in central and peripheral nervous system disorders and in controls. *Eur Neurol* 1998;**39**:103–10.
9. **Greenshields KN,** Halstead SK, Zitman FM, *et al*. The neuropathic potential of anti-GM1 autoantibodies is regulated by the local glycolipid environment in mice. *J Clin Invest* 2009;**119**:595–610.
10. **Yuki N,** Kuwabara S. Axonal Guillain-Barré syndrome: carbohydrate mimicry and pathophysiology. *J Peripher Nerv Syst* 2007;**12**:238–49.
11. **Uncini A,** Yuki N. Electrophysiologic and immunopathologic correlates in Guillain-Barré syndrome subtypes. *Expert Rev Neurother* 2009;**9**:869–84.
12. **Tatsumoto M,** Koga M, Gilbert M, *et al*. Spectrum of neurological diseases associated with antibodies to minor gangliosides GM1b and GalNAc-GD1a. *J Neuroimmunol* 2006;**177**:201–8.
13. **Kiernan MC,** Guglielmi JM, Kaji R, *et al*. Evidence for axonal membrane hyperpolarization in multifocal motor neuropathy with conduction block. *Brain* 2002;**125**:664–75.
14. **Priori A,** Bossi B, Ardolino G, *et al*. Pathophysiological heterogeneity of conduction blocks in multifocal motor neuropathy. *Brain* 2005;**128**:1642–8.
15. **Santoro M,** Uncini A, Corbo M, *et al*. Experimental conduction block induced by serum from a patient with anti-GM1 antibodies. *Ann Neurol* 1992;**31**:385–90.
16. **Harvey GK,** Toyka KV, Zielasek J, *et al*. Failure of anti-GM1 IgG or IgM to induce conduction block following intraneural transfer. *Muscle Nerve* 1995;**18**:388–94.
17. **Taylor BV,** Dyck PJ, Engelstad J, *et al*. Multifocal motor neuropathy: pathologic alterations at the site of conduction block. *J Neuropathol Exp Neurol* 2004;**63**:129–37.
18. **Jacobs BC,** O'Hanlon GM, Bullens RW, *et al*. Immunoglobulins inhibit pathophysiological effects of anti-GQ1b-positive sera at motor nerve terminals through inhibition of antibody binding. *Brain* 2003;**126**:2220–34.
19. **Zhang G,** Lopez PH, Li CY, *et al*. Anti-ganglioside antibody-mediated neuronal cytotoxicity and its protection by intravenous immunoglobulin: implications for immune neuropathies. *Brain* 2004;**127**:1085–100.
20. **Malik U,** Oleksowicz L, Latov N, *et al*. Intravenous gamma-globulin inhibits binding of anti-GM1 to its target antigen. *Ann Neurol* 1996;**39**:136–9.
21. **Basta M,** Dalakas MC. High-dose intravenous immunoglobulin exerts its beneficial effect in patients with dermatomyositis by blocking endomysial deposition of activated complement fragments. *J Clin Invest* 1994;**94**:1729–35.
22. **Basta M,** van Goor F, Luccioli S, *et al*. F(ab)'2-mediated neutralization of C3a and C5a anaphylatoxins: a novel effector function of immunoglobulins. *Nat Med* 2003;**9**:431–8.
23. **Lutz HU,** Stammler P, Bianchi V, *et al*. Intravenously applied IgG stimulates complement attenuation in a complement-dependent autoimmune disease at the amplifying C3 convertase level. *Blood* 2004;**103**:465–72.
24. **Lutz HU,** Stammler P, Jelezarova E, *et al*. High doses of immunoglobulin G attenuate immune aggregate-mediated complement activation by enhancing physiologic cleavage of C3b in C3bn-IgG complexes. *Blood* 1996;**88**:184–93.
25. **Kazatchkine MD,** Kaveri SV. Immunomodulation of autoimmune and inflammatory diseases with intravenous immune globulin. *N Engl J Med* 2001;**345**:747–55.
26. **Halstead SK,** Zitman FM, Humphreys PD, *et al*. Eculizumab prevents anti-ganglioside antibody-mediated neuropathy in a murine model. *Brain* 2008;**131**:1197–208.
27. **Phongsisay V,** Susuki K, Matsuno K, *et al*. Complement inhibitor prevents disruption of sodium channel clusters in a rabbit model of Guillain-Barré syndrome. *J Neuroimmunol* 2008;**205**:101–4.

JNNP Online First, published on November 7, 2010 as 10.1136/jnnp.2010.227777

LETTER

Lack of antibody response to Guillain–Barré syndrome-related gangliosides in mice and men after novel flu vaccination

During a mass vaccination campaign in the USA in 1976, there was a statistically significant increased risk of developing Guillain–Barré syndrome (GBS) following receipt of the A/NJ/1976/H1N1 'swine flu' vaccine.[1] Because the currently circulating pandemic A (H1N1) flu virus is partially of swine origin, there has been concern about a similar association of GBS with the novel flu A (H1N1) vaccine. Preliminary analysis showed an elevated, statistically significant association between 2009 H1N1 vaccination and GBS.[2] If confirmed, the increased risk of GBS associated with 2009 H1N1 vaccine of 0.8 cases per 1 million vaccinations would be comparable with the risk described previously for some trivalent seasonal flu vaccine formulations.

GBS is divided into two major subtypes, acute inflammatory demyelinating polyradiculoneuropathy (AIDP) and acute motor axonal neuropathy (AMAN).[3] AMAN, but not AIDP, is significantly associated with IgG antibodies against GM1, GM1b, GD1a, GalNAc-GD1a and GD1b. It is not known if the 1976 flu vaccine was associated with AIDP or AMAN. A recent report, however, demonstrated that the 1976 swine flu vaccines, seasonal flu vaccines from 1991–1992 and 2004–2005, and recombinant haemagglutinin proteins derived from high pathogenic avian H5N1 viruses A/HK/156/97 and A/Vietnam/1203/04 induced IgM and IgG anti-GM1 antibodies in mice.[4] Here, we report our assessment of the pandemic 2009 A (H1N1) and H5N1 vaccines' ability to induce antiganglioside antibodies in mice and humans, providing information as to the possible risk of developing AMAN following these vaccinations.

Inactivated A/H1N1pdm split vaccines (without adjuvant) used during the Japan 2009–2010 vaccination programme (A/California/7/2009 NYMC X-179A) were supplied by Kitasato Institute (Tokyo, Japan) and Denka Seiken (Tokyo, Japan). Inactivated, aluminium hydroxide-adjuvant H5N1 whole vaccines (A/Indo/05/2005-PR8-IBCDC-RG2, A/Anhui/01/2005-PR8-IBCDC-RG5 and A/Viet Nam/1194/2004-NIBRG-14) were provided by Research Foundation for Microbial Diseases of Osaka University (Biken) (Kagawa, Japan) and Kitasato Institute (Tokyo, Japan). Trivalent seasonal split vaccine (without adjuvant) of the Japan 2008–2009 vaccination programme (A/Brisbane/59/2007, A/Uruguay/716/2007 and B/Florida/4/2006) was supplied by Denka Seiken (Tokyo, Japan). Additional trivalent vaccine preparation used during the US 2004–2005 vaccination programme (A/New Caledonia/20/99, A/Wyoming/03/2003 and B/Jiangsu/10/2003) was kindly provided by Irving Nachamkin (University of Pennsylvania School of Medicine, Philadelphia). The 1976 swine flu vaccines were not available to study.

Mice lacking the functional gene for (*N*-acetylneuraminyl)-galactosylglucosylceramide *N*-acetylgalactosaminyltransferase (GalNAcT$^{-/-}$ mice) do not express complex gangliosides and are naïve hosts against ganglioside. In these mice, ganglioside-like lipo-oligosaccharide of a *Campylobacter jejuni* strain from AMAN elicits high titres of antiganglioside antibodies.[5] As previously described,[4] 7- to 10-week-old GalNAcT$^{-/-}$ mice were intramuscularly injected with the recommended adult human dose (0.5 ml, equivalent to 15 μg of haemagglutinin) of vaccine 3 weeks apart, whereas 9-week-old C3H/HeN mice were subcutaneously injected (box 1). Serum samples were obtained before each immunisation and 2 weeks after the second immunisation. Experimental protocols were approved by Animal Care and Use Committees.

Neither IgM nor IgG antibodies against GM1, GM1b, GD1a, GalNAc-GD1a, GD1b, GT1a and GQ1b were detected in GalNAcT$^{-/-}$ mice vaccinated with A/H1N1pdm vaccine (Denka Seiken) (n=10), H5N1 vaccines (A/Indo/05/2005-PR8-IBCDC-RG2 (n=5), A/Anhui/01/2005-PR8-IBCDC-RG5 (n=5) and A/Viet Nam/1194/2004-NIBRG-14 (n=5)), trivalent seasonal vaccines from Japan 2008–2009 (n=5) and the US 2004–2005 vaccination programmes (n=5). No antiganglioside antibodies were induced in C3H/HeN mice inoculated with A/H1N1pdm vaccine (Denka Seiken) (n=5), H5N1 vaccine (A/Indo/05/2005-PR8-IBCDC-RG2) (n=5) or trivalent seasonal vaccines from Japan 2008–2009 vaccination programme (n=5), whereas serum haemagglutination inhibition titres increased from <10 to 80±49 after inoculation of A/H1N1pdm vaccine. Despite the use of the same seasonal 2004–2005 flu vaccine and C3H/HeN mice,[4] we could not confirm earlier observations that these flu vaccines elicit an antiganglioside antibody response. Moreover, no antiganglioside antibodies were induced in the naïve mice. The previous study did not describe whether optical densities of GM1-free wells were subtracted from densities of GM1-coated wells,[4] raising the possibility that non-specific IgM and IgG responses were shown.

A total of 200 eligible subjects underwent randomisation to receive 15 μg of haemagglutinin antigen (A/H1N1pdm split vaccine, Kitasato Institute) subcutaneously or 30 μg intramuscularly. They had previously received two doses of the assigned vaccine 3 weeks apart in 2009 (box 1). A total of 121 eligible subjects were administered 15 μg of haemagglutinin antigen (whole H5N1 vaccines; A/Viet Nam/1194/2004-NIBRG-14, Biken or A/Anhui/01/2005-PR8-IBCDC-RG5A, Kitasato Institute) adjuvanted with alum, who previously received two subcutaneousdoses of the assigned vaccine 3 weeks apart in 2008. Serum samples were obtained from each subject before each vaccination, 3 weeks after the second vaccination or 6 months after the first vaccination. A total of 137 eligible subjects were administered 5 or 15 μg of haemagglutinin antigen (whole H5N1 vaccine; A/Indo/05/2005-PR8-IBCDC-RG2, Biken) intramuscularly 3 weeks apart in 2006, then received 15 μg of haemagglutinin antigen (whole H5N1 vaccines; A/Viet Nam/1194/2004-NIBRG-14 or A/Anhui/01/2005-PR8-IBCDC-RG5A) in 2008. Serum samples were obtained before the second vaccination, and 1 and 3 weeks after the second vaccination. Informed written consent was obtained from each subject.

Box 1

(A) Mouse immunisation
- GalNAcT$^{-/-}$ mice 15 μg im, 3 weeks apart
- C3H/HeN mice 15 μg sc, 3 weeks apart.

(B) Human vaccination
- A/H1N1pdm split vaccines.
  - 15 μg sc, two doses, 3 weeks apart in 2009 (n=100).
  - 30 μg im, two doses, 3 weeks apart in 2009 (n=100).
- Whole H5N1 vaccines.
  - 15 μg sc, two doses, 3 weeks apart in 2008 (n=121).
  - 5 or 15 μg im, two doses 3 weeks apart in 2006 and 15 μg im, one dose in 2008 (n=137).

IgM anti-GM1 antibodies and low-affinity IgG anti-GM1 antibodies are induced in non-diseased rabbits sensitised with GM1 or in AMAN rabbits before the onset.[3] This raises a possibility that such low-affinity anti-GM1 antibodies were induced in some of human subjects, although none of those developed GBS. Both IgM and IgG antibodies against GM1, GM1b, GD1a, GalNAc-GD1a, GD1b, GT1a and GQ1b were undetectable in sera from the 200 subjects who received A/H1N1pdm vaccine twice, whereas they obtained high titres of neutralising antibodies against the vaccine-strain flu virus as well as haemaglutination inhibition (http://www.mhlw.go.jp/bunya/kenkou/kekkaku-kansenshou04/infuI_iken-koukan1111.html). The aforementioned antiganglioside antibodies were also not induced in 258 subjects who received H5N1 vaccine, although the neutralising antibodies and haemaglutination inhibition activities against the vaccine virus were present (Ihara, Ito, Kobayashi and Kamiya, in preparation).

The flu vaccines studied here elicited no antiganglioside antibody response in mice

(n=50) and men (n=458). A thin-layer chromatogram with resorcinol staining did not detect gangliosides in any of the vaccines. Previous haemagglutinin inhibition findings suggested the presence of a GM1 epitope on flu viruses,[4] but our immunoblot results failed to detect haemagglutinin-associated GM1. In conclusion, our results suggest that the flu vaccines are unlikely to induce the production of antiganglioside antibodies associated with AMAN.

Acknowledgements We thank JJ Sejvar (Centers for Disease Control and Prevention, Atlanta) for his scientific discussions and editing; T Nakano (Mie National Hospital), S Hattori (Mie Chuou Medical Center), K Horibe (Nagoya Medical Center), A Shimatsu (Kyoto Medical Center), Y Saeki (Osaka Minami Medical Center), K Terayama (Kyushu Medical Center), H Suzuki (Suzuka Kaisei Hospital), K Ohe (Konan Kosei Hospital), T Ozaki (Konan Kosei Hospital) and H Kakitoh (Mie University Hospital) for obtaining informed consents from participants; Y Okuno (Research Foundation for Microbial Diseases of Osaka University), K Takasugi (Denka Seiken) and I Nachamkin (University of Pennsylvania School of Medicine) for providing the vaccines; H Watanabe (Niigata National Hospital), M Funakoshi (Dokkyo Medical University), C Yanaka (Dokkyo Medical University), S Koike (Dokkyo Medical University) and A Koguchi (Dokkyo Medical University) for their technical assistance; and M Tashiro (National Institute of Infectious Diseases) and S Tomizawa (Niigata National Hospital) for their critical reading; N Shahrizaila (University of Malaya) for her editing.

Nobuhiro Yuki,[1,2] Yoshimasa Takahashi,[3] Toshiaki Ihara,[4] Suminobu Ito,[5] Takashi Nakajima,[1] Kei Funakoshi,[6] Koichi Furukawa,[7] Kazuo Kobayashi,[3] Masaaki Odaka[6]

[1]Departments of Neurology and Clinical Research, Niigata National Hospital, National Hospital Organization, Niigata, Japan; [2]Departments of Microbiology and Medicine, National University of Singapore, Singapore; [3]Department of Immunology, National Institute of Infectious Diseases, Tokyo, Japan; [4]Department of Pediatrics, Mie National Hospital, National Hospital Organization, Mie, Japan; [5]Department of Clinical Research, Clinical Research Center, National Hospital Organization Headquarters, Tokyo, Japan; [6]Department of Neurology, Dokkyo Medical University, Tochigi, Japan; [7]Department of Biochemistry II, Nagoya University School of Medicine, Nagoya, Japan

Correspondence to Dr Nobuhiro Yuki, Departments of Microbiology and Medicine, National University of Singapore, Science Drive 2, Blk MD4A, Level 5, 117597 Singapore; micyuki@nus.edu.sg

Competing interests None.

Patient consent Obtained.

Ethics approval All relevant documents were approved by the ethical review committee at each hospital.

Provenance and peer review Not commissioned; externally peer reviewed.

Received 22 August 2010
Revised 26 September 2010
Accepted 28 September 2010

J Neurol Neurosurg Psychiatry 2010;■:1–2.
doi:10.1136/jnnp.2010.227777

REFERENCES

1. **Lehmann HC,** Hartung HP, Kieseier BC, *et al.* Guillain–Barré syndrome after exposure to influenza virus. *Lancet Infect Dis* 2010;**10**:643–51.
2. **Centers for Disease Control and Prevention (CDC).** Preliminary results: surveillance for Guillain–Barré syndrome after receipt of influenza A (H1N1) 2009 monovalent vaccine—United States, 2009–2010. *MMWR Morb Mortal Wkly Rep* 2010;**59**:657–61.
3. **Yuki N,** Kuwabara S. Axonal Guillain–Barré syndrome: carbohydrate mimicry and pathophysiology. *J Peripher Nerv Syst* 2007;**12**:238–49.
4. **Nachamkin I,** Shadomy SV, Moran AP, *et al.* Anti-ganglioside antibody induction by swine (A/NJ/1976/H1N1) and other influenza vaccines: insights into vaccine-associated Guillain–Barré syndrome. *J Infect Dis* 2008;**198**:226–33.
5. **Matsumoto Y,** Yuki N, van Kaer L, *et al.* Guillain–Barré syndrome-associated IgG responses to gangliosides are generated independently of CD1 function in mice. *J Immunol* 2008;**180**:39–43.