The Lower Serum Immunoglobulin G2 Level in Severe Cases than in Mild Cases of Pandemic H1N1 2009 Influenza Is Associated with Cytokine Dysregulation

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The majority of patients with pandemic influenza H1N1 2009 had mild illness, but some, including those with no risk factors for severe disease, may succumb to this infection. Besides viral factors such as the D222/225G substitution of the hemagglutinin, host factors such as IgG2 subclass deficiency recently was reported to be associated with severe disease in a cohort of Australian patients besides other known risk factors, including underlying chronic illness, extremes of age, and pregnancy. We conducted a case-control study of 38 Asian patients with respiratory failure due to severe pandemic influenza and compared the results to those for 36 mild cases. None had selective IgG2 deficiency, but the level of IgG2 subclass was significantly lower in the severe cases (3.55 g/liter versus 4.75 g/liter; P = 0.002), whereas the levels of IgG1, IgG3, and IgG4 were not significantly different from those of the mild cases. Previous studies suggested that some IgHG2 and FcγRIIa genotypes were associated with IgG2 deficiency. The allelic frequency of the IgHG2 genotypes in our severe cases was not correlated with their levels of IgG2, while that of FcγRIIa was not significantly different from that of the general Han Chinese population (P = 0.216). Only the overall cytokine/chemokine profile (P = 0.029) and serum globulin level (P = 0.005) were found to be independently associated with the IgG2 level by multivariate analysis. The lower IgG2 level in our severe group might be related to cytokine dysregulation rather than being a significant risk factor for severe pandemic influenza. The importance of this finding for therapeutic intervention will require further studies of larger cohorts of patients.

The first pandemic of the 21st century, caused by the 2009 H1N1 influenza virus, has affected millions of patients and caused more than 18,449 deaths in more than 214 countries worldwide between its onset in March 2009 and its official step down to the postpandemic phase on 10 August 2010 (http://www.who.int/csr/don/2010_08_06/en/index.html). Although the majority of cases had mild clinical presentations, many severe or even fatal cases involved young patients without known risk factors, such as pregnancy, obesity, extremes of age, chronic illnesses, and immunosuppression. In some of these cases, the explanation may be related to viral factors, including the ability of the virus to replicate in human epithelial cells of the lower respiratory tract (10), the D222/225G substitution (3, 13, 31) in the viral hemagglutinin, and perhaps its resistance to the lower respiratory tract (10), the D222/225G substitution (3, 13, 31) in the viral hemagglutinin, and perhaps its resistance to

39 patients in Victoria, Australia, which was one of the key areas for the pandemic in the southern hemisphere (6). Although a significant proportion of the patients with severe infection in this cohort were found to be IgG2 deficient, it was unclear whether the deficiency was the underlying predisposing factor or the result of host-virus interaction. We attempted to look for this immunodeficiency among the patients in Hong Kong, where outbreaks of various types of influenza continue to be a major public health concern (23, 26, 29, 30), by comparing the IgG2 levels between severe and mild cases of pandemic influenza in the locality. To determine the significance of the difference in IgG2 levels between the two groups, we further correlated their IgG2 levels with their clinical and laboratory findings. We then searched for genetic factors that would affect the IgG2 level by assessing their IgHG2 (7) and FcγRIIa genotypes (22), and we evaluated the effect of the overall cytokine/chemokine profile on the IgG2 level during the infection.

MATERIALS AND METHODS

The study was approved by the Institutional Review Board of the Hospital Authority in Hong Kong.

Patients. A total of 74 patients who were admitted to hospitals in Hong Kong for pandemic influenza H1N1 2009 between May 2009 and January 2010 were included in the study. The severe group included 38 patients who required...
respiratory support or admission to the intensive care unit for respiratory decompensation, and the mild group included 36 randomly selected patients who did not develop respiratory decompensation. Thirty-two of these 74 patients, 18 in the severe group and 14 in the mild group, were reported previously (25). The diagnosis was confirmed by the detection of the pandemic H1 gene in reverse transcription-PCR (RT-PCR) or viral culture in nasopharyngeal or endotracheal specimens, as previously described (15). Clinical data, including history, physical examination findings, and results of hematological, biochemical, radiological, and microbiological investigations, were retrieved from a retrospective review of medical records and entered into a predesigned database. All cases were observed until death or discharge from the hospital.

Cytokine and chemokine profile assay. The Milliplex human cytokine/chemokine kit premixed 24-plex (Millipore, MA) was used to determine the plasma cytokine and chemokine levels according to the manufacturer’s instructions and as previously described (25, 27).

IgG subclass levels. The BINDARID human IgG subclass single-dilution kit (The Binding Site Ltd., Birmingham, AL) was used to determine the IgG subclass levels according to the manufacturer’s instructions. In brief, for IgG1 and IgG2 testing, a 1:10 dilution was made from mixing 25 μl of patient serum with 225 μl of 7% bovine serum albumin (BSA). No dilution was made for IgG3 and IgG4, as recommended. Each well in the radial immunodiffusion (RID) plates was filled with 5 μl of neat calibrator, medium calibrator dilution, low calibrator dilution sample, or control. The plates were incubated at 20°C for 72 h. The diameters of the precipitin rings were measured to the nearest 0.1 mm using an RID reader, and the squares of the diameters were used to construct a calibration curve. The IgG subclass concentration was determined from the calibration curve.

IgHG2 genotyping. IgHG2 genotyping was performed by adapting the method defined by Hougs et al. (7). Genomic DNA was extracted from peripheral blood leukocytes with a QIAamp DNA blood mini kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. The following PCR primers were used: F1, 5′-TGAGCCCGCACACTGGA; R1, 5′-TGGCCACTGCACACA ACA; F2, 5′-AGAGCCGAAATGTTGTGT; R2, 5′-TGTCCATGTGGCCTCT ATA; F3, 5′-AGAGCCGAAATGTTGTGT; and R3, 5′-AGTGTTGGGAGCA CAG TGGAA. The PCR mixture (25 μl) contained denatured human genomic DNA, PCR buffer (10 mM Tris-Cl, pH 8.3, 50 mM KCl, 2 mM MgCl2, and 0.01% gelatin), 200 μM each deoxynucleoside triphosphate, and 0.625 U AmpliTaq gold polymerase (Applied Biosystems, Foster City, CA). Hot-start PCR was performed using the following conditions: min at 95°C, 40 cycles of 95°C for 1 min, 50°C (58°C for primers F1/R1 and F3/R3) for 1 min, and 72°C for 90 s, with a final extension at 72°C for 10 min in an automated thermal cycler (Applied Biosystems, Foster City, CA). Five microliters of each amplified product was electrophoresed in a 2.5% (wt/vol) agarose gel with a molecular size marker (50-bp DNA ladder; Fermentas, Canada) in parallel. Electrophoresis in Tris-borate-EDTA buffer was performed at 100 V for 1 h. The gel was stained with ethidium bromide (0.5 μg/ml) for 15 min, rinsed, and photographed under UV light illumination. The PCR product was gel purified using a QIAquick PCR purification kit (Qiagen, Hilden, Germany). Both strands of the PCR product were sequenced with an ABI prism 3130xl DNA analyzer according to the manufacturer’s instructions (Applied Biosystems, Foster City, CA), using the PCR primers. Thirty-seven of 38 patients in the severe group had blood samples available for testing.

FcγRIIa genotyping. FcγRIIa genotyping was performed by using the method defined by Osborne et al. (19). Primers were designed by multiple alignments using the ectodomains of FcγIIa, FcγIib, and FcγIic. The following PCR primers were used: F1, 5′-ACAGTCTCCCTAGGGTTAT; R1, 5′-ACCTAACAACAG CTGAGAAG; Fii, 5′-TGTGAACTACGCACCTACGG; and Rii, 5′-CCAGT GCAAATTTGCT. The PCR mixture and conditions were the same as those described above. Thirty-seven of 38 patients in the severe group had blood samples available for testing, whereas all 36 patients in the mild group were tested.

Statistical analysis. Clinical, virological, and immunological characteristics were compared. The Fisher exact test and χ² test were used for categorical variables where appropriate, and the Mann-Whitney U test was used for continuous variables because the data were not normally distributed. Multivariate analysis was performed for finding factors independently associated with the IgG2 level among age, pregnancy status, lymphocyte count, albumin level, globulin level, FcγRIIa allotype, and overall cytokine/chemokine profile. Principal component analysis was used to reduce the profile of the cytokines to the first principal component. SPSS software, version 17.0 for Windows, was used for statistical computation. A P of <0.050 was considered to represent a statistically significant difference.

RESULTS

The severe and mild groups were matched by age and sex. They had no significant difference in terms of demographic characteristics, comorbidities, and symptomatology, except for the development of respiratory distress at presentation (Table 1). They had comparable hematological and biochemical parameters, except that the severe group had a lower absolute lymphocyte count and albumin level (Table 2). The severe group developed more complications, including a requirement for mechanical ventilation, admission to the intensive care unit, acute respiratory distress syndrome (ARDS), multigorgan dysfunction syndrome (MODS), and death, and it received more aggressive therapy, including double-dose oseltamivir and intravenous and nebulized/inhaled zanamivir (Table 2). Although the difference between the two groups in terms of the occurrence of bacterial coinfections did not reach statistical significance (P = 0.154), the majority were in the severe group (8/10; 80%) (Table 3). All of them developed pulmonary infections in terms of pneumonia or empyema. Six of them had infection due to Staphylococcus aureus, and the remaining four patients had infection due to Streptococcus pneumoniae, group G streptococcus, Pseudomonas aeruginosa, or Haemophilus influenzae. In terms of their immunological responses, the severe group had significantly lower IgG2 levels than the mild group (Table 4). This finding held true even if the cutoff value of the Australian cohort was applied (in the current study, the normal range was ≥1.65 g/liter; in reference 6, the normal range was ≥2.40 g/liter). The IgG1, IgG3, and IgG4 levels of the two groups did not differ significantly. Higher levels of granulocyte colony-stimulating factor (G-CSF), interleukin-1α (IL-1α), IL-6, IL-8, IL-10, IL-15, IP-10, monocyte chemotactic protein 1 (MCP-1), and tumor necrosis factor alpha (TNF-α) were observed in the severe group.

As for the genotypes of IgHG2, 18 of 37 (48.6%) patients in

| Parameter | Severe cases (n = 38) | Mild cases (n = 36) | P value |
|-----------|----------------------|---------------------|--------|
| Age, median yr (range) | 49.0 (20.0–84.0) | 39.5 (20.0–83.0) | 0.058 |
| Sex, male/female | 21/17 | 22/14 | 0.610 |
| Pregnancy (%) | 1 (2.6) | 2 (5.6) | 0.610 |
| Smoking (%) | 9 (23.7) | 8 (22.2) | 0.881 |
| Drinking (%) | 6 (15.8) | 5 (13.9) | 0.818 |
| Underlying disease | | | |
| No underlying disease (%) | 20 (52.6) | 21 (58.3) | 0.647 |
| Chronic cardiac disease (%) | 4 (10.5) | 6 (16.7) | 0.510 |
| Chronic pulmonary disease (%) | 9 (23.7) | 6 (16.7) | 0.453 |
| Chronic liver disease (%) | 1 (2.6) | 3 (8.3) | 0.351 |
| Chronic renal disease (%) | 2 (5.3) | 1 (2.8) | 1.000 |
| Diabetes mellitus (%) | 6 (15.8) | 5 (13.9) | 0.818 |
| Malignancy (%) | 4 (10.5) | 4 (11.1) | 1.000 |
| Clinical presentation | | | |
| Feverishness (%) | 30 (78.9) | 29 (80.1) | 0.863 |
| Cough (%) | 33 (86.8) | 26 (72.2) | 0.118 |
| Sputum (%) | 24 (63.2) | 16 (44.4) | 0.106 |
| Sore throat (%) | 11 (28.9) | 14 (38.9) | 0.366 |
| Wheezing (%) | 2 (5.3) | 1 (2.8) | 0.494 |
| Malignancy (%) | 8 (21.1) | 10 (27.8) | 0.500 |
| Dyspnea (%) | 32 (84.2) | 5 (13.9) | <0.001 |
| Vomiting (%) | 6 (15.8) | 4 (11.1) | 0.737 |
| Diarrhea (%) | 1 (2.6) | 3 (8.3) | 0.351 |

TABLE 1. Demographic and clinical characteristics
the severe group were n+/n+, 14 (37.8%) were n+/n−, and 5 (15.3%) were n−/n− (Table 5). Our patients’ genotypes were significantly different from those of the Japanese and Dan-ish Caucasian general populations (7), in that our patients had a much higher proportion of n+/n+ (48.6% in this study, 2.2% in the Japanese population, and 20.3% in the Danish Caucasian population) and less n−/n− (13.5% in this study, 71.4% in the Japanese population, and 27.9% in the Danish Caucasian population). For the genotypes of FcRIIa, 23 (62.2%) of 37 severe cases were H131H, 9 (24.3%) were H131R, and 5 (15.5%) were R131R. In the mild group, 16 of 36 (44.4%) cases were H131H, 17 (47.2%) were H131R, and 3 (8.3%) were R131R (Table 5). This was not significantly different from the general Han Chinese population (P = 0.216) (http://www.hapmap.org). The association between the expected allotypes (i.e., H131H as “FcRIIa-H131” and R131R and H131R as “FcRIIa-R131”) (19) and the level of IgG2 was not statistically significant in multivariate analysis.

Besides FcRIIa allotype, other factors, including age, pregnancy status, lymphocyte count, and albumin level, were not found to be significantly associated with the IgG2 level in multivariate analysis. The two factors that were significantly associated with the level of IgG2 were the overall cytokine/chemokine profile (P = 0.029) and the serum globulin level (P = 0.005). To determine the significance of the overall cytokine/chemokine profile, principal combined analysis was used to reduce the profile of the individual cytokines/chemokines to the first principal component before applying multivariate analysis. Component one represented 28.8% of the total variance.

**DISCUSSION**

Our study has shown that Asian patients with a more severe clinical manifestation of pandemic influenza had a relatively lower overall level of IgG2 than patients with milder presentation. This was similar to the previously reported observation among Australian patients. While this key finding was consis-

| Parameter | Severe cases (n = 36) | Mild cases (n = 36) | P value |
|-----------|----------------------|--------------------|---------|
| Initial laboratory findings on admission, median (range) | | | |
| Total white blood cell count, ×10^9 cells/liter | 6.45 (0.80–32.05) | 6.25 (3.60–13.59) | 0.978 |
| Neutrophil count, ×10^9 cells/liter | 5.05 (0.40–30.45) | 4.50 (2.20–10.73) | 0.314 |
| Lymphocyte count, ×10^9 cells/liter | 0.60 (0.20–2.10) | 0.90 (0.37–2.60) | <0.001 |
| Hemoglobin, g/dl | 12.60 (6.90–20.90) | 13.60 (9.90–16.70) | 0.189 |
| Platelet count, ×10^9 cells/liter | 180.00 (68.00–312.00) | 179.00 (79.00–289.00) | 0.430 |
| Alanine transaminase level, IU/liter | 31.00 (10.00–261.00) | 22.50 (10.00–116.00) | 0.115 |
| Albumin, g/liter | 32.00 (22.00–47.00) | 41.00 (27.00–50.00) | <0.001 |
| Globulin, g/liter | 32.50 (21.00–53.00) | 32.00 (21.00–47.00) | 0.965 |
| Creatinine, mol/liter | 88.50 (46.00–497.00) | 79.00 (45.00–261.00) | 0.247 |

**TABLE 2. Investigations, treatments, complications, and clinical outcomes**

| Antiviral treatment (%) | % | % |
|-------------------------|---|---|
| Osmeltamivir (standard or double dose) | 36 (94.7) | 32 (88.9) | 0.424 |
| Standard dose | 14 (36.8) | 32 (88.9) | <0.001 |
| Double dose | 22 (57.9) | 0 (0.0) | <0.001 |
| Zanamivir (intravenous, nebulized, or inhaled) | 17 (44.7) | 1 (2.8) | <0.001 |
| Intravenous | 7 (18.4) | 0 (0.0) | 0.012 |
| Nebulized/inhaled | 10 (26.3) | 1 (2.8) | 0.007 |

| Complications and clinical outcome (%) | % | % |
|---------------------------------------|---|---|
| Mechanical ventilation | 32 (84.2) | 0 (0.0) | <0.001 |
| ICU admission | 35 (92.1) | 0 (0.0) | <0.001 |
| Bacterial coinfection | 8 (21.1) | 2 (5.6) | 0.154 |
| ARDS | 25 (65.8) | 0 (0.0) | <0.001 |
| MODS | 28 (73.7) | 0 (0.0) | <0.001 |
| Death | 14 (36.9) | 0 (0.0) | <0.001 |

**TABLE 3. Patients with bacterial coinfection, their respective IgG2 level, and IgHG2 and FcRIIa genotypes**

| Patient | Severity | Bacterial coinfection^a (clinical syndrome) | IgG2 level (g/liter) | IgHG2 genotype | FcRIIa genotype |
|---------|----------|--------------------------------------------|----------------------|----------------|-----------------|
| 1       | Severe   | Streptococcus pneumoniae (bacteremic pneumonia) | 1.70                 | Not available | R131R           |
| 2       | Severe   | MRSA (bacteremic empyema)                   | 2.50                 | n+/n+         | H131H           |
| 3       | Severe   | Group G streptococcus (pneumonia)          | 2.50                 | n+/n−         | H131H           |
| 4       | Severe   | MSSA (bacteremic pneumonia)                | 2.80                 | n−/n−         | H131H           |
| 5       | Severe   | MSSA (pneumonia)                           | 3.40                 | n+/n+         | H131H           |
| 6       | Severe   | MSSA (pneumonia)                           | 4.30                 | n+/n+         | H131H           |
| 7       | Severe   | Pseudomonas aeruginosa (pneumonia)         | 4.70                 | n−/n−         | H131H           |
| 8       | Severe   | MSSA (pneumonia)                           | 6.80                 | n+/n+         | H131R           |
| 9       | Mild     | MSSA (pneumonia)                           | 3.40                 | Not available | H131R           |
| 10      | Mild     | Haemophilus influenzae (pneumonia)         | 4.40                 | Not available | H131H           |

^a MSSA, methicillin-sensitive *Staphylococcus aureus*; MRSA, methicillin-resistant *Staphylococcus aureus*. 
tent, there were some notable differences in the other findings of the two cohorts. In the Australian cohort, not only did the severe group have a lower overall IgG2 level but the majority of the individual patients (16/19; 84.2%) with severe infection also had IgG2 deficiency (6). Among our patients, only 1 out of 38 patients (2.6%) with severe infection had IgG2 deficiency. Even if the cutoff level of 2.4 g/liter used in the Australian study was adopted for our patients, only a minority of patients with severe infection (4/38; 10.5%) would fall into the “deficient” category. Also, the only patient in our severe group with deficiency did not have selective IgG2 deficiency but did have a broad spectrum of deficiencies also affecting IgG1 and IgG3. This might be another difference between the two cohorts, although the details of the IgG3 level were not described in the material.

Various factors may affect the IgG2 level either permanently or transiently. To determine whether the relatively lower overall IgG2 level found in our severe cases was indeed the underlying cause of the critical illness or merely an indicator of severe disease, we assessed their genetic factors, including the IgH2G2 and FcγRIIa genotypes, and acquired factors, including age, pregnancy status, lymphocyte count, albumin level, globulin level, and cytokine/chemokine profile, using multivariate analysis.

While homozygous deletions of the corresponding C region genes have been shown to be the underlying mechanism of IgG2 deficiency (18), most cases remain largely idiopathic and may be related to an aberrant regulation of the expression of the IgH2G2 genes. The three genotypes, namely, n+/n+, n+/n−, and n−/n−, were found to be related to IgG2 levels (7). In patients with IgG2 deficiency, the n−/n− genotype was found in more than 50% of cases (20). In our severe group, which had an overall lower IgG2 level, the predominant genotype was n+/n+ (48.6%), while only 13.5% of them had the n−/n− genotype. This was quite different from previous findings. Because of this reason, and as there were no comparable data available from the Chinese general population, IgH2G2 genotyping was not performed for the mild group, as we considered that the correlation of IgH2G2 genotypes with IgG2 level was weak in our population.

The other important genetic factor that is closely related to IgG2 is FcγRIIA. Fcγ receptors are an important link between the humoral and cellular immune systems. The efficacy of IgG-induced leukocyte effector functions, including antibody-dependent cellular cytotoxicity, phagocytosis, superoxide generation, degranulation, cytokine production, and the regulation

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### TABLE 4. Initial plasma IgG and cytokine/chemokine levels

| Parameter | Severe cases (n = 38) | Mild cases (n = 36) | P value |
|-----------|----------------------|---------------------|---------|
| IgG1 level, g/liter | 6.55 (1.10–18.00) | 6.70 (3.50–12.30) | 0.713 |
| IgG1, median (range) | 3.55 (1.10–8.00) | 4.75 (2.00–7.95) | 0.002 |
| IgG1, median (range) | 0.83 (0.15–4.60) | 0.86 (0.32–3.30) | 0.689 |
| IgG4, median (range) | 0.54 (0.06–1.85) | 0.95 (0.14–3.65) | 0.087 |

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### TABLE 5. IgH2G2 and FcγRIIa genotypes

| Genotype | Severe cases (%) (n = 37) | Mild cases (%) (n = 36) |
|----------|--------------------------|-------------------------|
| IgH2G2 | n+/n+ | 18 (48.6) | Not available |
| n+/n− | 14 (37.8) | Not available |
| n−/n− | 5 (13.5) | Not available |
| FcγRIIa | H131H | 23 (62.2) | 16 (44.4) |
| H131R | 9 (24.3) | 17 (47.2) |
| R131R | 5 (13.5) | 3 (8.3) |

*a One patient in the severe group had combined IgG1, IgG2, and IgG3 deficiency.
of antibody production, shows interindividual heterogeneity due to genetic polymorphisms of the Fcγ receptor subclasses. Among them, FcγRIIa is the only receptor capable of binding IgG2 efficiently. FcγRIIa polymorphism is due to a G-to-A point mutation in the region specifying its ligand-binding domain, which results in an arginine (R)-to-histidine (H) amino acid alteration at position 131. The FcγRIIIa-H131 allotype has higher binding efficiency for IgG2 and greater phagocytic capability for IgG2-opsonized bacteria than the FcγRIIa-R131 allotype. A recent study by Endeman et al. showed that the FcγRIIa-R131R genotype was found more frequently in patients with severe community-acquired pneumonia caused by *S. pneumoniae* and *H. influenzae* (5). In our patients with bacterial coinfections, this association was not obvious (Table 3). Importantly, individuals with the FcγRIIIa-H131 allotype have been shown to possess lower levels of IgG2 than individuals with the FcγRIIa-R131 allotype (22). However, this association was not observed in our patients (Table 5). We believe that while FcγRIIa polymorphism may have a firmly established qualitative association with IgG2, their quantitative relationship requires further investigations.

In our cohort, only the overall cytokine/chemokine profile (*P* = 0.029) and the serum globulin level (*P* = 0.005) were found to be significantly associated with the IgG2 level. Similarly to previous reports (1, 25), the severe group had a marked cytokine response, with various pro- and anti-inflammatory cytokines being significantly raised. The overall effect was cytokine dysregulation, which had both antiviral activity and damage on host tissue, leading to acute respiratory distress syndrome. The cytokine regulation of IgG2 production is still a largely ambiguous subject. Gamma interferon (IFN-γ) may have a role, as suggested in previous studies (9, 12, 14). However, we were unable to note any significant difference in IFN-γ level between the mild and severe groups in our study. Thus, we believe that in the case of severe pandemic influenza, a more probable explanation of low IgG2 levels is the more prominent Th1 response and a relatively suppressed Th2 response, as reported previously (1).

Although definitive causation cannot be determined by our findings, the absence of selective IgG2 deficiency in our severe cases, the close association between the IgG2 level and the cytokine/chemokine profile, and the lack of association between the IgG2 level and genetic factors in terms of the IgHG2 and FcγRIIa genotypes suggest that relative IgG2 suppression represents the result of cytokine dysregulation rather than a significant predisposing risk factor for the pandemic influenza, whereas selective IgG2 deficiency is a rare phenomenon in Asian Chinese with severe pandemic influenza. However, this does not preclude the possibility that low levels of IgG2 increase the subsequent risk of bacterial coinfection.

Our hypothesis has important therapeutic implications in the management of severe influenza. Once infection occurs, early antiviral therapy with a neuraminidase inhibitor to reduce viral load (2, 16) is crucial, as a high viral load is associated with the subsequent development of cytokine dysregulation (24, 27). In those who present late where cytokine dysregulation has been established, the option of immunomodulation by hyperimmune intravenous immunoglobulin may be considered (25, 28). Even though low IgG2 levels may be observed in critically ill patients, the attempt to increase its level by the administration of IFN-γ could not be recommended from our findings, as we believe that it is not an important underlying cause of severe illness. Though the use of convalescent-phase plasma or hyperimmune intravenous immunoglobulin with neutralizing antibody against the pandemic virus may be considered in severe illness (8, 17), the main purpose is for the rapid reduction of viral load. Additional benefit from protection against encapsulated bacterial coinfection results from the associated IgG2 subclass replacement. Besides the neuraminidase inhibitors, new antiviral agents targeting the nucleoprotein may become a useful treatment in the future (11).

In conclusion, our study is the first and largest one to describe the association between low IgG2 levels and severe pandemic influenza in Asians, among whom outbreaks of influenza continue to be a major public health concern. While IgG2 deficiency may have a role as a risk factor for severe pandemic influenza in the Australian population, the absence of selective IgG2 deficiency in our severe cases, the association between the IgG2 level and the cytokine/chemokine profile, and the lack of association between the IgG2 level and genetic factors make this less conclusive in our population. Alternatively, the role of the lower overall level of IgG2 as part of an overall cytokine dysregulation commonly observed in severe influenza appears to be a more probable explanation. Future studies focusing on the exact mechanism of the cytokine regulation of IgG2 should be conducted to confirm this hypothesis and generate the potentially useful therapy of cytokine dysregulation in severe influenza.

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