donors (Fig 2, *A* and *B*). Patients with PGM3^{Glu340del}, known to cause a severe phenotype, had less expression of gp130 than did those with PGM3^{Leu83Ser} (Fig 2, *A* and *B*), a finding attributed to the more profound impairment of glycosylation caused by the p.Glu340del mutation.[4] *PGM3* gene silencing will definitely further confirm the role of PGM3-dependent glycosylation in gp130 signaling. We also assessed the effect of tunicamycin, an inhibitor of N-glycosylation, on gp130-mediated signaling in EBV cell lines from a healthy donor. Tunicamycin treatment gradually replaced the glycosylated form of gp130 (~130 kDa) with its unglycosylated form (100 kDa) and inhibited STAT3 activation in response to IL-6 (Fig 2, *C* and *D*). The absence of the lower 100-kD band, corresponding to the unglycosylated gp130 isoform in the patients' lysates (Fig 2, *B*), is likely due to proteasomal degradation, as has been seen in another study.[7] Our results are consistent with the observation that N-glycosylation inhibition of gp130 abolishes IL-6–driven STAT3 activation in cultured cardiac myocytes.[8]

Collectively, our findings demonstrate that defective glycosylation in PGM3-deficient patients results in reduced expression of unglycosylated gp130 protein and consequently, impaired gp130-dependent STAT3 phosphorylation. This may account for the overlapping clinical features shared by PGM3 deficiency, AD-HIES, and gp130 deficiency. Indeed, we have shown that PGM3-deficient patients have defective IL-6 and IL-27 signaling. Impaired IL-6/gp130/STAT3 signaling in PGM3-deficient patients could explain in part low IL-17–producing T cells generated *in vitro* as well as low memory B cells. In addition, the impairment of T-cell proliferation and the increased proportion of T_H2 cells observed in PGM3-deficient patients could be attributed to aberrant IL-27 signaling. Indeed, several reports showed that IL-27 plays a critical role in the suppression of T_H2 responses.[9] Altogether, these functional cellular phenotypes observed in PGM3-deficient patients overlap with STAT3 loss of function and gp130-deficient patients and mechanistically tie the PGM3 deficiency to poor gp130-dependent STAT3 signaling.

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Antibody response to common human viruses is shaped by genetic factors

To the Editor:

Significant interindividual variation exists among "normal" human humoral immune responses to viral infection. However, although the genetic etiology of immunologic extremes such as primary immunodeficiency has been well characterized, little is known about the genetics of normal interindividual variation in immune response. The classical twin design, which compares the phenotypic similarity between monozygotic (MZ) twin pairs and dizygotic (DZ) twin pairs, can be used to estimate the proportion of interindividual variation due to genetic factors. For example, the classical twin design has been used to demonstrate that antibody response to vaccination against certain pathogens is likely to be influenced by genetic factors.[1] The present study used twin and sibling data to estimate the genetic and environmental determinants of antibody titers to 6 common human viruses: EBV, Coxsackie B virus (CVB), parvovirus B-19 (PV-B19), herpes simplex virus 1 (HSV-1), human herpes virus 6 (HHV-6), and cytomegalovirus (CMV). Although these viruses usually cause relatively mild symptoms or are asymptomatic, many are also observationally associated with the development of more severe diseases, including autoimmune diseases such as multiple sclerosis, rheumatoid arthritis, type 1 diabetes, and systemic lupus erythematosus.[2]

Participants were recruited as part of the Brisbane Adolescent Twin Sample (also known as the Brisbane Longitudinal Twin Study) conducted at the QIMR Berghofer Medical Research Institute (QIMRB).[3] Zygosity was determined using whole-genome genotyping using single nucleotide polymorphism chips. Ethical approval for the study was obtained from the QIMRB Human Research Ethics Committee, and informed consent was obtained from all participants and their guardians. Blood samples were collected from study participants (including

TABLE I. Medians with lower (Q1) and upper (Q3) quartiles for raw antibody titers against common human viruses in seropositive adolescent twins and siblings

Variable	Units	Seroprevalence* (%)	Median†	Q1-Q3†	N†
EBV	U/mL	45.1	58.22	38.99-74.51	828
CVB	U/mL	42.3	27.32	19.81-43.43	776
PV-B19	U/mL	33.1	95.48	48.78-186.10	608
HSV-1	Index	21.3	5.39	3.02-7.36	391
HHV-6	Index	81.4	3.45	2.46-4.50	1494
CMV	U/mL	50.7	72.96	38.46-116.70	931

*Unadjusted overall seroprevalence of common human viruses in adolescents (N = 1835).
†Seropositive individuals only.

TABLE II. Correlations between MZ and DZ twin pairs and sibling-twin correlations for seropositive quantitative antibody traits and serostatus

A. Titer

Variable	Twin correlation				Sibling-twin correlation (95% CI)		Proportion of total variance							
	MZ (95% CI)		DZ (95% CI)				a^2 (95% CI)		c^2 (95% CI)		d^2 (95% CI)		e^2 (95% CI)	
EBV	0.67	0.53 to 0.76	0.32	0.17 to 0.45	0.14	−0.05 to 0.31	0.28	0.00 to 0.70	—	—	0.39	0 to 0.76	0.33	0.24 to 0.47
CVB	0.55	0.36 to 0.68	0.01	−0.20 to 0.18	0.12	−0.04 to 0.27	0.00	0.00 to 0.31	—	—	0.52	0.15 to 0.66	0.48	0.34 to 0.68
PV-B19	0.63	0.44 to 0.74	0.66	0.53 to 0.75	0.21	−0.05 to 0.41	0.44	0.11 to 0.73	0.20	(0-0.44)	—	—	0.36	0.25 to 0.52
HSV-1	0.54	0.17 to 0.72	0.40	0.11 to 0.59	0.50	0.27 to 0.64	0.15	0.00 to 0.67	0.38	(0-0.60)	—	—	0.47	0.28 to 0.69
HHV-6	0.50	0.37 to 0.60	0.31	0.10 to 0.40	0.15	0.04 to 0.25	0.37	0.05 to 0.57	—	—	0.14	0 to 0.50	0.49	0.39 to 0.61
CMV	0.78	0.69 to 0.84	0.46	0.33 to 0.56	0.40	0.25 to 0.52	0.69	0.47 to 0.83	0.08	0-0.27	—	—	0.22	0.16 to 0.31

B. Serostatus

Variable	Twin correlation				Sibling-twin correlation (95% CI)		Proportion of total variance							
	MZ (95% CI)		DZ (95% CI)				a^2 (95% CI)		c^2 (95% CI)		d^2 (95% CI)		e^2 (95% CI)	
EBV	0.79	0.67 to 0.88	0.78	0.70 to 0.85	0.66	0.55 to 0.75	0.16	0 to 0.37	0.64	0.48 to 0.78	—	—	0.20	0.12 to 0.30
CVB	0.46	0.28 to 0.62	0.24	0.09 to 0.38	0.15	0.00 to 0.28	0.29	0 to 0.55	—	—	0.17	0 to 0.61		
	0.38	to 0.71												
0.54														
PV-B19	0.93	0.87 to 0.97	0.88	0.81 to 0.92	0.83	0.74 to 0.88	0.17	0.03 to 0.30	0.77	0.65 to 0.86	—	—	0.06	0.03 to 0.13
HSV-1	0.88	0.78 to 0.94	0.86	0.78 to 0.92	0.68	0.55 to 0.79	0.22	0.02 to 0.41	0.67	0.51 to 0.81	—	—	0.11	0.04 to 0.20
HHV-6	0.67	0.48 to 0.81	0.57	0.41 to 0.69	0.39	0.20 to 0.56	0.37	0 to 0.73	0.31	0.03 to 0.57	—	—	0.32	0.19 to 0.51
CMV	0.87	0.79 to 0.93	0.83	0.75 to 0.89	0.61	0.49 to 0.71	0.32	0.15 to 0.48	0.57	0.43 to 0.70	—	—	0.11	0.06 to 0.19

twins and siblings within 5 years of their age) following the 12th birthday of the twin pair, or as near as could be arranged (median age, 12.2 years; N = 1835 individuals consisting of 231 MZ and 435 DZ twin pairs and their similar aged siblings).

Five milliliters of venous blood were collected, and serum was extracted after centrifugation. Serum was frozen and stored at −80°C using previously described protocols that do not impact antibody detection with time.[4] The samples were then thawed and ELISA was performed to measure IgG antibody levels against EBV (anti-EBNA-1; Genway Biotech, San Diego, Calif), CVB (anti-B1, B3, B5; Institut Virion Serion, Wurzberg, Germany), PV-B19 (Institut Virion Serion), HSV-1 (Fortress Diagnostics, Antrim, United Kingdom), HHV-6 (Abnova, Taipei City, Taiwan), and CMV (Fortress Diagnostics). Plates were read using a Biotek PowerWave HT Microplate Spectrophotometer. Absorbance values were converted into units (U/mL or positivity index) and dichotomous serostatus phenotypes were determined according to manufacturer instructions for each ELISA kit—antibody levels above a calibrated threshold for detectability were classified as seropositive, whereas those below the threshold were seronegative. Table I presents the seroprevalence and median and interquartile ranges of seropositive individuals for each of the 6 antibodies.

Inverse normalization was used to transform quantitative antibody levels in seropositive individuals for each virus before analysis. Titers were residualized after adjusting for batch effects (ie, for plate, row, and column of the sample) and were used as the dependent variables in the variance components models. Structural equation modeling using the software program Mx[5] was used to partition variance into possible sources of genetic and environmental variation. Analysis of serostatus used a liability threshold model and all models used full information maximum likelihood as the fit function. Means (or thresholds) and variances were constrained equally across all participants for each antibody. Common environment shared by twin pairs was assumed to be equal to common environment shared by all family members. All models included sex and age as fixed effects.

In the case of antibody titers, point estimates of the correlation between MZ twins appeared to be higher than point estimates of the correlation between DZ twins in the case of all viruses with the exception of PV-B19, suggesting a role for genetics in explaining interindividual variation in antibody titer (Table II). In contrast,

point estimates of the MZ and DZ correlations appeared much more similar for serostatus, with only CVB having substantially different MZ and DZ correlations, suggesting a major role for the common environment in explaining interindividual variation in serostatus for most of the viruses we studied. Correlations between related individuals were generally higher for serostatus than for quantitative antibody measure, signifying that families often share exposure to viruses but not necessarily their extent of immune response. DZ twins and sibling-twin pairs share the same amount of genetic information on average, but correlation estimates differed between the groups. However, for most viruses, the CIs around the DZ and sibling-twin correlations were overlapping. Substantial differences between DZ twin pair and sibling-twin correlations were observed for quantitative PV-B19 as well as CMV serostatus. The fact that MZ and DZ twin correlations were similar but sibling-twin correlations were generally lower for serostatus could be indicative of shared environment specific to twins, which may arise from twins being treated more similarly than regular siblings or because individuals of the same age are more likely to share a common environment.

Variance components analysis of the quantitative and serostatus data revealed significant heritability for most traits (Table II). Estimates of the common environmental variance component were generally not significant for quantitative antibody titer (ie, 95% CIs overlapping 0) but significant for serostatus. Estimates of the unique environmental component were lower for serostatus than for quantitative titer. These results imply that genetics influences the extent of response more than viral susceptibility, and conversely that the common environment accordingly appears to influence susceptibility more than viral response. Considering that serostatus at least partially reflects exposure to the pathogen and technical parameters of the assay (such as limit of detection), substantial common environmental estimates may be expected. Our heritability estimates for response to the herpes-viruses (HSV-1, HHV-6, CMV, and EBV) are within the CIs reported in a previous article that measured antibody response to these viruses in a large Mexican family study,[6] although this previous study analyzed titers from seropositive and seronegative individuals, which may help explain some of the differences in estimates between our studies.

Levels of antibody titers to CVB was the only trait for which dominance genetic effects appeared to be significant (ie, 95% CIs for the dominance genetic effects, d^2, did not span 0). Interestingly, this implies that the extent of IgG response to CVB maybe unlike that of the other viruses. This may be because CVB is the only RNA enterovirus among the 6 measured and is also the only virus with characteristically acute infection.[7] IgG levels against CVB may vary over time,[8] and if timing of infection is genetically controlled, then this might increase MZ correlations relative to other familial correlations and give the appearance of dominance effects.

We have presented the results of our analyses as point estimates and 95% CIs of the proportion of trait variance explained by additive (a^2) and dominance genetic factors (d^2), and common (c^2) and unique environmental factors (e^2). Variance components that are statistically significant from zero have 95% CIs that do not overlap the null (0). Although our statistical comparisons would not survive correction for multiple testing using a stringent Bonferroni threshold (eg, $\alpha = 0.05/(6 \text{ viruses} \times 2 \text{ (serostatus/titer)})$, the number of variance components in our analyses where the CIs do not overlap 0 is far greater than what would be expected by chance. This suggests that most of our findings regarding heritability and the common environmental are likely to be real. Replication in much larger and more powerful twin studies will be necessary to confirm our results. In conclusion, we found that the extent of antibody response to 6 common human viruses was influenced by genetic factors—as was viral susceptibility to a lesser extent. Common environment influenced the extent of response but had a larger effect on viral susceptibility. Our demonstration of significant heritability for antibody response represents the first step in the eventual identification of individual genetic variants that influence the phenotype using genome-wide association. Each of the viruses we have examined in this article is observationally associated with the development of 1 or more autoimmune diseases. Our intention is to use individual genetic variants that we discover from future genome-wide association studies as genetic instruments to investigate possible causal relationships between infection by these viruses and development of autoimmune disease using Mendelian randomization approaches.[9]

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A case of mistaken identity: The MAR-1 antibody to mouse FcεRIα cross-reacts with FcγRI and FcγRIV

To the Editor:

IgE is a primary driver of allergic symptoms mediated by cells expressing the high-affinity Fc receptor for IgE, FcεRI. The cell types expressing FcεRI and downstream pathways have been extensively studied in mouse models of allergic disease. The MAR-1 mAb is thought to specifically recognize the α chain of mouse FcεRI. Although FcεRI was initially thought to be uniquely expressed by basophils and mast cells in mice,[1] several recent studies using the MAR-1 antibody for flow cytometric analysis reported that FcεRI is expressed on dendritic cells (DCs), particularly monocyte-derived dendritic cells (moDCs), in mice under various inflammatory conditions, such as exposure to house dust mite (HDM), the bacterial cell-wall component LPS, or Sendai virus.[2-4] The MAR-1 antibody has also been injected into mice for numerous functional studies, including basophil depletion[5] and rapid desensitization.[6] Surprisingly, several results obtained with MAR-1–mediated basophil depletion have not been recapitulated in genetic models of basophil depletion.[5] These unexpected findings based on MAR-1 prompted us to re-examine the specificity of this antibody.

We first sought to clarify whether moDCs express FcεRI under inflammatory conditions. Mice were treated intranasally with HDM extract and examined 3 days later by using flow cytometric analysis with the MAR-1 antibody (see Methods and Table E1 in this article's Online Repository at www.jacionline.org). We were able to identify FcεRIα$^+$ CD11c$^+$ MHC class II$^+$ CD11b$^+$ inflammatory moDCs in the lungs and mediastinal lymph nodes (mLNs), but we were surprised to find that these apparent FcεRIα$^+$ moDCs were also present in FcεRIα-deficient mice (*Fcer1a*$^{-/-}$ mice, hereafter denoted as FcεRIα KO; Fig 1, *A*, and see Fig E1 in this article's Online Repository at www.jacionline.org). Because the MAR-1 antibody labeled these cells in the absence of FcεRIα expression, we reasoned it would be more appropriate to call these MAR-1$^+$ moDCs.

Because MAR-1$^+$ moDCs have also been reported in mice with viral infection,[3] we next tested MAR-1 staining of moDCs in this setting. To mimic viral infection, we administered intranasal polyinosinic:polycytidylic acid (poly I:C), a Toll-like receptor 3 ligand, and also detected MAR-1$^+$ moDCs in the lungs. Similar to HDM exposure, we found that MAR-1$^+$ moDCs could also be identified in poly I:C–treated FcεRIα KO mice similar to control mice (Fig 1, *B*). In contrast, blood basophils only stained with MAR-1 from control mice but not from FcεRIα KO mice (Fig 1, *C*), confirming the genotype of the FcεRIα KO mice.

We next considered whether the MAR-1 antibody might bind to other Fc receptors and stained cells from mice lacking the Fc receptor common γ chain (*Fcer1g*$^{-/-}$ mice, hereafter denoted as FcRγc KO), which is necessary for normal surface expression of all activating Fc receptors. In FcRγc KO mice, MAR-1 staining was greatly diminished on moDCs from the lungs and mLNs after HDM treatment (Fig 1, *D*). Macrophages and monocytes express various Fcγ receptors but are not known to express FcεRI in mice, yet one study noted MAR-1 staining on monocytes.[6] We tested whether MAR-1 would stain splenic red pulp macrophages, lung alveolar macrophages, peritoneal macrophages, and blood monocytes. Indeed, MAR-1 stained all of these macrophages (Fig 1, *E*) and a subset of blood Ly6C$^-$ and Ly6C$^+$ monocytes in both wild-type and FcεRIα KO mice (Fig 1, *F*). Similar to moDCs, MAR-1 staining was greatly reduced in these populations in FcRγc KO mice. These observations imply that MAR-1 might be cross-reacting with other Fc receptors, thereby resulting in detection of MAR-1$^+$ cells in FcεRIα KO mice.

Because our findings above suggested that MAR-1 binds one of the activating Fcγ receptors, we next attempted to identify to which Fc receptors MAR-1 might bind. In mice the activating Fc receptors that bind IgG are FcγRI (CD64), FcγRIII (CD16), and FcγRIV (CD16-2). We noticed that MAR-1 staining strongly correlated with FcγRI (CD64) staining on moDCs (Fig 2, *A*) and that MAR-1 staining correlated with FcγRIV (CD16-2) staining on Ly6C$^-$ blood monocytes (Fig 2, *B*).

To definitively test whether MAR-1 was cross-reacting with FcγRI (CD64) and FcγRIV (CD16-2), we expressed specific FcγRs in a cell line and then stained with MAR-1 versus other FcγR-specific antibodies. Specifically, the Phoenix cell line, a modified 293T human embryonic kidney cell line, was individually transfected with the α chains of FcγRI, FcγRIII, or FcγRIV together with the Fc receptor common γ chain and then stained with antibodies. Untransfected cells did not stain with MAR-1 or any of the activating FcγR antibodies, as expected, confirming that this human cell line lacks endogenous reactivity with these mouse reagents (Fig 2, *C*). In support of our observations in monocytes and macrophages, Phoenix cells transfected individually with FcγRI or FcγRIV stained with the MAR-1 antibody (Fig 2, *C*). In contrast, MAR-1 did not stain FcγRIII-transfected cells (Fig 2, *C*), demonstrating that MAR-1 binds to the α chains of FcγRI and FcγRIV but not the shared Fc receptor common γ chain.

We next confirmed that the MAR-1 staining on myeloid cells *in vivo*, in the absence of FcεRI, was due to binding to FcγRI and FcγRIV. We analyzed splenocytes from mice deficient in the α chains of all 4 FcγRs (FcγRα 4 KO), which showed an absence of MAR-1 staining on splenic red pulp macrophages and monocytes (see Fig E2 in this article's Online Repository at www.jacionline.org). Neutrophils, which are known to express FcγRIV,[7] also stained weakly with MAR-1 in control but not in FcγRα 4 KO mice (see Fig E2). Some residual surface FcγRIV expression was also noted in the FcRγc KO mice compared with the FcγRα 4 KO mice (see Fig E2), providing a potential explanation for the weak MAR-1 staining observed on some cell populations in the FcRγc KO mice. Although our findings do not formally exclude the possibility that MAR-1 might bind additional receptors, taken together, our data indicate that MAR-1 binding to FcγRI and FcγRIV accounts for most of the MAR-1 staining on macrophages, monocytes, and neutrophils. Similar MAR-1 staining was observed on splenocytes from wild-type B6 and BALB/c mice, indicating our findings are broadly applicable (see Fig E3 in this article's Online Repository at www.jacionline.org).