The phase III RV144 HIV-1 vaccine trial estimated vaccine efficacy (VE) to be 31.2%. This trial demonstrated that the presence of HIV-1–specific IgG-binding Abs to envelope (Env) V1V2 inversely correlated with infection risk, while the presence of Env-specific plasma IgA Abs directly correlated with risk of HIV-1 infection. Moreover, Ab-dependent cellular cytotoxicity responses inversely correlated with risk of infection in vaccine recipients with low IgA; therefore, we hypothesized that vaccine-induced Fc receptor–mediated (FcR-mediated) Ab function is indicative of vaccine protection. We sequenced exons and surrounding areas of FcR-encoding genes and found one FCGR2C tag SNP (rs114945036) that associated with VE against HIV-1 subtype CRF01_AE, with lysine at position 169 (169K) in the V2 loop (CRF01_AE 169K). Individuals carrying CC in this SNP had an estimated VE of 15%, while individuals carrying CT or TT exhibited a VE of 91%. Furthermore, the rs114945036 SNP was highly associated with 3 other FCGR2C SNPs (rs138747765, rs78603008, and rs373013207). Env-specific IgG and IgG3 Abs, IgG avidity, and neutralizing Abs inversely correlated with CRF01_AE 169K HIV-1 infection risk in the CT- or TT-carrying vaccine recipients only. These data suggest a potent role of Fc-γ receptors and Fc-mediated Ab function in conferring protection from transmission risk in the RV144 VE trial.
FCGR2C polymorphisms associate with HIV-1 vaccine protection in RV144 trial


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Introduction
The Thai phase III RV144 vaccine trial, which tested the ALVAC-HIV (vCP1521) prime and bivalent clade B/E recombinant gp120 boost vaccine regimen, showed an estimated vaccine efficacy (VE) of 31.2% for prevention of HIV-1 infection (1). This result provided an opportunity to search for immune correlates of protection and to study viral and host genetics to understand better the predictors and mechanisms of vaccine protection. Studies of immune responses as correlates of acquisition risk (CoRs) of HIV-1 infection showed that plasma IgG-binding Abs to scaffolded gp70-V1V2 proteins inversely correlated with infection risk and specific HIV-1 envelope (Env) gp120 plasma IgA-binding Abs directly correlated with infection risk (2–4). IgG avidity, Ab-dependent cellular cytotoxicity (ADCC), neutralizing Abs (NAbs), and CD4+ T cell responses inversely correlated with infection risk in the subgroup of vaccinees with low IgA; and specific Env IgA/IgG ratios directly correlated with HIV-1 infection risk in all vaccinees, indicating that Env-specific IgA Abs may have blocked protective IgG Fc-mediated effector functions (5).

Part of the biological activities of Abs results from interactions between the Fc region of Abs and Fc receptors (FcRs) on cells, including monocytes, macrophages, dendritic cells, neutrophils, and NK cells. Fc-γ receptors (FcγRs) play multifaceted roles in immune complexes: they control innate immune effector cell activation, regulate the production and specificity of Abs, regulate B cell activation and plasma cell survival, and function in antigen presentation and immune complex–mediated maturation of dendritic cells (6). Recent studies have demonstrated the crucial role of FcγRs widely expressed on innate immune effector cells in

Authorship note: Shuying S. Li and Peter B. Gilbert contributed equally to this work.

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mediating the protective functions of IgG (7). Aberrant expression or the presence of certain allelic variations of FcγRs is associated with altered functionality that in turn is associated with susceptibility to, or increased severity of, certain autoimmune and infectious diseases and to outcomes of monoclonal Ab cancer treatments (8–17). Besides IgG Abs, IgA Abs also play an important role in humoral immunity. IgA interacting with IgA receptors (particularly FcαR) has been found to affect pathogenesis of diseases and autoimmune conditions (18). However, immunogenetic variation of FcγRs and FcαRs in immune responses to and clinical protection by vaccines in humans remains under investigated. The correlates of binding Ab responses with HIV-1 infection risk in RV144, the importance of Fc-FcR interactions to Ab function, and differential expression of FcR genotypes led us to determine whether FcR polymorphisms were related to the VE observed in RV144.

Results

Description of FcR SNP genotypes. We used amplicon resequencing technology to sequence the external protein domains and the transmembrane regions of the 5 low-affinity FcγR genes (FCGR2A, FCGR2B, FCGR2C, FCGR3A, FCGR3B) and the complete coding region of the FcαR gene (FCAR) (Supplemental Figure 1 and Supplemental Table 1; supplemental material available online with this article; doi:10.1172/JCI75539DS1). These sequences were obtained for the 125 HIV-1-infected subjects (51 vaccine and 74 placebo) and the 205 uninfected vaccine recipient controls used in the case-control correlates analysis (2). Sequencing resulted in genotypes of 148 FCGR SNPs (Supplemental Table 2A) and 42 FCAR SNPs (Supplemental Table 3A). To reduce the number of tests and increase statistical power, we restricted the analysis to a subset of SNPs that passed quality control based on Hardy-Weinberg equilibrium (P > 0.00001), had enough variability to be able to potentially detect an association with VE (minor allele frequency greater than 5%), and were not highly correlated with any other SNP (maximum Pearson correlation with any other SNP r² < 0.8). Using the tag SNP selection algorithm implemented in Haploview (19, 20), we selected 28 tag SNPs from the 55 SNPs that satisfied the first 2 screening criteria for further analysis (Supplemental Table 2 for FCGRs and Supplemental Table 3 for FCAR). If a tag SNP significantly associated with VE, the SNPs that were captured by the tag SNP (correlated with r² ≥ 0.8) would be evaluated, and the tag SNP and captured SNPs would be annotated for interpretations.

Association of FcRs with VE against HIV-1 acquisition. Using the case-only method based on the 125 HIV-1-infected cases (51 vaccine and 74 placebo) (21), none of the 28 tag SNPs were significantly associated with VE against HIV-1 infection after family-wise error rate (FWER) multiplicity correction (Supplemental Table 4). Previous reports showed that the estimated VE against infection with HIV-1 of subtype CRF01_AE 169K that matched the vaccine insert at position 169 (169K) was relatively high at 48% (22), and the vaccine regimen induced Abs specific for 169K in the V2 loop (3, 23). Therefore, we also assessed associations of the Fcγ receptors with VE against CRF01_AE 169K HIV-1 (Supplemental Table 4). Based on the 87 CRF01_AE 169K HIV-1-infected cases (30 vaccine and 57 placebo), one tag SNP (rs114945036) significantly associated with VE against HIV-1 infection after family-wise error rate (FWER) multiplicity correction (Supplemental Table 4). The estimated VEs for the subjects who carried the CC and CT/TT genotypes of FCGR2C 126C>T were 64% for CT/TT genotypes of FCGR2C 126C>T (rs114945036) genotype with VE against any HIV-1 strain and VE against CRF01_AE 169K HIV-1. The error bars are 95% confidence intervals for VE.
TT subjects in both time periods (VE against any HIV-1 strain was reduced from 27% to –10% in CC and from 73% to 50% in CT/TT individuals; VE against CRF01_AE 169K HIV-1 was reduced from 41% to –36% in CC and from 100% to 78% in CT/TT individuals) (Supplemental Table 5).

Our further investigations focused on FCGR2C 126C>T as a marker for the SNPs that significantly associated with VE. We addressed 3 follow-up questions. (a) Did the previously identified differential VE against HIV genotypes with amino acid signatures at Env V2 positions 169 and 181 differ between CC and CT/TT subjects? (b) Did the immune responses to vaccination at week 26 differ between CC and CT/TT vaccine recipients? (c) Did the association of immune responses with HIV-1 infection risk differ in CC and CT/TT vaccine recipients?

Increased VE against CRF01_AE HIV-1 matched to the vaccine at position 169 was restricted to the CT/TT subgroup. Previous sieve analysis identified 2 HIV-1 signature sites at Env V2 positions 169 and 181, wherein VE was significantly greater against HIV-1 matched compared with HIV-1 mismatched at site 169, and VE was significantly greater against HIV-1 mismatched than matched to the vaccine at site 181 (22). Detection of such differential VE at a signature site provides evidence that the site is involved in an immune mechanism of vaccine protection. Therefore, the previous analysis of differential VE against HIV-1 genotypes defined by match or mismatch at sites 169 and 181 was repeated within the CC and CT/TT subgroups. As shown in Figure 2A, differential VE at position 169 was observed in CT/TT subjects (VE = 91% against 169 matched vs. VE < 0% against 169 mismatched HIV-1, P value for differential VE = 0.007) but not in CC subjects (P value for differential VE = 0.45), with interaction P = 0.041 for the differential VE against any HIV-1 strain was reduced from 27% to –10% in CC and from 73% to 50% in CT/TT individuals; VE against CRF01_AE 169K HIV-1 was reduced from 41% to –36% in CC and from 100% to 78% in CT/TT individuals) (Supplemental Table 5).

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Accordingly, the remaining analyses of the FCGR2C 126C>T SNP involving the HIV-1 sequence data focus on the 169 signature site.

Second, we assessed whether FCGR2C 126C>T was associated with immune responses measured 2 weeks after the last vaccination (week 26 visit). As shown in Figure 3 and Supplemental Figures 2–4, distributions of these week 26 immune response measures stratified by FCGR2C VE being restricted to (or stronger in) the CT/TT subgroup. These results support that the vaccine selectively blocked infection with CRF01_AE 169K HIV-1 only among CT/TT subjects, generating the hypothesis that a mechanism of protection involving site 169 only occurs in individuals with a CT or TT genotype.

In contrast, there was no evidence that the differential VE at position 181 occurred more or less strongly in the CC subgroup compared with the CT/TT subgroup (Figure 2B, interaction P = 0.99).

Accordingly, the remaining analyses of the FCGR2C 126C>T SNP involving the HIV-1 sequence data focus on the 169 signature site.

No association of the FCGR2C 126C>T genotype with vaccine-elicited immune responses. Second, we assessed whether FCGR2C 126C>T was associated with immune responses measured 2 weeks after the last vaccination (week 26 visit). As shown in Figure 3 and Supplemental Figures 2–4, distributions of these week 26 immune response measures stratified by FCGR2C
126C>T genotype in the 205 uninfected vaccine recipient controls and the 2 vaccine recipient–infected case groups (i.e., all 41 HIV-1–infected cases and the 25 CRF01_AE 169K HIV-1–infected cases). None of the immune responses significantly differed between the CC and CT/TT vaccine recipient controls (all P > 0.05), indicating that the FCGR2C 126C>T genotype did not seem to affect the ability of the HIV vaccine to induce the immune responses.

FCGR2C 126C>T genotype as an effect modifier of correlates of risk of HIV-1 infection — 6 primary and 8 sensitivity variables and 1 secondary variable assessed originally in RV144. Third, we assessed whether FCGR2C 126C>T was an effect modifier of the correlation of immune responses measured at week 26 with the risk of HIV-1 infection by 42 months as well as with the risk of CRF01_AE 169K HIV-1 infection by 42 months. For risk of infection with any HIV-1 strain, the correlation for any of the 15 variables did not significantly differ between the CC and CT/TT vaccine recipient subgroups (interaction test P > 0.05, q > 0.2) (Table 1). However, there was a nonsignificant trend (interaction P = 0.13) for the primary IgA Env–binding variable to directly correlate with HIV-1 risk in the CC vaccine recipients (odds ratio [OR] = 1.53 per 1-SD increase, P = 0.001, respectively, whereas for CT/TT vaccine recipients OR = 0.09 per 1-SD increase, P = 0.45 and P = 0.91). Similarly, the IgA C1 peptide and IgA consensus A gp140 variables directly correlated with HIV-1 infection risk only in the CC vaccine recipients (OR = 1.74 and OR = 1.72 per 1-SD increase, P = 0.0005 and P = 0.0005, respectively, whereas for CT/TT vaccine recipients OR < 0.01 per 1-SD increase, P > 0.05). For risk of infection with CRF01_AE 169K HIV-1, the correlation of IgG avidity and NAbs with infection risk differed between CC and CT/TT vaccine recipients at marginal significance levels (interaction test q = 0.20) (IgG avidity: OR = 0.87 per 1-SD increase, P = 0.55 in CC vs. OR = 0.09 per 1-SD increase, P = 0.05 in CT/TT; NAbs: OR = 1.23 per 1-SD increase, P = 0.36 in CC vs. OR < 0.01 per 1-SD increase).
increase, \( P = 0.061 \) in CT/TT). Similar trends were observed for the corresponding avidity and NAb sensitivity variables (Table 1). Similar to the overall HIV-1 infection results, the direct correlation of IgA Env binding with risk of CRF01_AE 169K HIV-1 infection appeared restricted to the CC vaccine recipients (Table 1).

FCGR2C 126C>T genotype as an effect modifier of correlates of risk of HIV-1 infection — IgG response to V1V2 scaffold antigens and to Env gp120s. The magnitudes of Abs reactive with subtype A, B, and C and CRF01_AE V1V2 scaffold antigens were shown previously to correlate inversely with HIV-1 infection risk (4). These inverse correlations were not significantly modified by FCGR2C 126C>T (all interaction \( P > 0.05 \)), although the estimated ORs of CRF01_AE 169K HIV-1 infection tended to be lower in CT/TT vaccine recipients than CC vaccine recipients (Supplemental Table 6). We also assessed the IgG breadth score to the V1V2 scaffold antigens as a CoR in all vaccinees and in the 2 FCGR2C 126C>T genotype subgroups of vaccinees, in which the breadth score for a vaccinee is defined as the median of the IgG response magnitudes to the 11 V1V2 antigens. The IgG V1V2 breadth score was inversely correlated with overall HIV-1 infection risk in all vaccine recipients (OR = 0.66, \( P = 0.013 \)), but this correlation was not significantly modified by FCGR2C 126C>T genotype (Supplemental Table 6). There was a trend toward a stronger inverse CoR of CRF01_AE 169K HIV-1 infection in the CT/TT vaccine recipients (overall OR = 0.60, \( P = 0.006 \); CC OR = 0.63, \( P = 0.026 \); CT/TT OR = 0.26, \( P = 0.027 \)) (Supplemental Table 6). Last, the inverse correlations of the IgG responses to the 2 evaluated Env antigens (the vaccine insert strain A244 and a circulating strain from RV144 [AE703357]) with CRF01_AE 169K HIV-1 infection risk were restricted to the CT/TT vaccine recipients (Table 2, interaction \( P = 0.053 \) and \( P = 0.063 \), respectively). Therefore, these HIV-1 Env antigens likely express relevant epitopes that are also presented by HIV-1–infected cells and are important for Fc-FcR Ab-mediated effector function.

FCGR2C 126C>T genotype as an effect modifier of correlates of risk of HIV-1 infection — IgG3 response to V1V2 scaffold antigens and to Env gp120s. The magnitudes of subclass IgG3 responses to the same V1V2 scaffold antigens measured for IgG did not correlate with risk of HIV-1 infection without adjusting for IgA in previous analyses (26). However, subclass IgG3 has been reported to bind with higher specificity and affinity than IgG1 to most FcγRs (27). Here, we

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### Table 2. Assessment of FCGR2C 126C>T (rs114945036) genotype modification of the correlation between IgG Ab binding to 2 Env gp120s (magnitude) at week 26 and risk of infection by 42 months among the vaccine recipients

<table>
<thead>
<tr>
<th>HIV-1 Env antigen</th>
<th>Genotype</th>
<th>No. of cases/controls</th>
<th>OR</th>
<th>( P ) value</th>
<th>Int. ( P ) value</th>
<th>No. of cases/controls</th>
<th>OR</th>
<th>( P ) value</th>
<th>Int. ( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>gp120s A244</td>
<td>CC</td>
<td>34:154</td>
<td>0.96</td>
<td>0.889</td>
<td>23:154</td>
<td>0.95</td>
<td>0.860</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CT/TT</td>
<td>7:49</td>
<td>0.64</td>
<td>0.079</td>
<td>0.272</td>
<td>2:49</td>
<td>0.40</td>
<td>0.009</td>
<td>0.053</td>
</tr>
<tr>
<td>gp120s AE703357</td>
<td>CC</td>
<td>34:154</td>
<td>1.06</td>
<td>0.843</td>
<td>23:154</td>
<td>0.97</td>
<td>0.908</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CT/TT</td>
<td>7:49</td>
<td>0.65</td>
<td>0.061</td>
<td>0.199</td>
<td>2:49</td>
<td>0.43</td>
<td>0.009</td>
<td>0.063</td>
</tr>
</tbody>
</table>

All infections with HIV-1 and CRF01_AE 169K HIV-1 were considered. Correlation was measured using the OR of risk probability per 1-SD increase in binding intensity in log10 scale. \(^a\)Interaction \( P \) value for testing a lack of a differential correlate of immune response with risk between the CC vaccine recipients and the CT/TT vaccine recipients. \( P \) values less than or equal to 5% appear in bold.

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### Table 3. Assessment of FCGR2C 126C>T (rs114945036) genotype modification of the correlation between IgG3 Ab binding to 3 V1V2 scaffold proteins and 2 Env gp120s (magnitude) at week 26 and the risk of infection by 42 months among the vaccine recipients

<table>
<thead>
<tr>
<th>HIV-1 V1V2 and Env antigen</th>
<th>Genotype</th>
<th>No. of cases/controls</th>
<th>OR</th>
<th>( P ) value</th>
<th>Int. ( P ) value</th>
<th>No. of cases/controls</th>
<th>OR</th>
<th>( P ) value</th>
<th>Int. ( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>gp70.AE(92TH023)-V1V2.AP</td>
<td>CC</td>
<td>34:154</td>
<td>0.98</td>
<td>0.921</td>
<td>23:154</td>
<td>0.96</td>
<td>0.850</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CT/TT</td>
<td>7:49</td>
<td>0.70</td>
<td>0.294</td>
<td>0.406</td>
<td>2:49</td>
<td>0.27</td>
<td>0.029</td>
<td>0.051</td>
</tr>
<tr>
<td>gp70.B(Case A2/mut3)-V1V2.LL</td>
<td>CC</td>
<td>34:154</td>
<td>0.99</td>
<td>0.946</td>
<td>23:154</td>
<td>0.96</td>
<td>0.871</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CT/TT</td>
<td>7:49</td>
<td>0.69</td>
<td>0.284</td>
<td>0.385</td>
<td>2:49</td>
<td>0.27</td>
<td>0.031</td>
<td>0.052</td>
</tr>
<tr>
<td>gp70.C(1086)-V1V2.LL</td>
<td>CC</td>
<td>34:154</td>
<td>0.99</td>
<td>0.970</td>
<td>23:154</td>
<td>0.96</td>
<td>0.852</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CT/TT</td>
<td>7:49</td>
<td>0.66</td>
<td>0.254</td>
<td>0.336</td>
<td>2:49</td>
<td>0.21</td>
<td>0.032</td>
<td>0.048</td>
</tr>
<tr>
<td>gp70.C AE703357</td>
<td>CC</td>
<td>34:154</td>
<td>1.12</td>
<td>0.615</td>
<td>23:154</td>
<td>0.97</td>
<td>0.905</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CT/TT</td>
<td>7:49</td>
<td>0.61</td>
<td>0.160</td>
<td>0.142</td>
<td>2:49</td>
<td>0.28</td>
<td>0.034</td>
<td>0.053</td>
</tr>
</tbody>
</table>

All infections with HIV-1 and CRF01_AE 169K HIV-1 were considered. Correlation was measured using the OR of risk probability per 1-SD increase in binding intensity in log10 scale. \(^a\)Interaction \( P \) value for testing a lack of a differential correlate of immune response with risk between the CC vaccine recipients and the CT/TT vaccine recipients. \( P \) values less than or equal to 5% appear in bold.
found that FCGR2C 126C>T genotype did not significantly modify the correlation of any of the 11 V1V2-specific IgG3 responses with overall HIV-1 infection risk (interaction test \( P > 0.05 \), Supplemental Table 7); however, there was borderline significant modification of the correlation of IgG3 responses to 3 V1V2-specific antigens with CRF01_AE 169K HIV-1 infection risk (Table 3; interaction \( P = 0.048 \) to \( P = 0.052 \)). In addition, the IgG3 V1V2 breadth score was not significantly correlated with overall HIV-1 infection risk in all vaccine recipients (OR = 0.79), and this correlation was not significantly modified by FCGR2C 126C>T genotype (Supplemental Table 7). The IgG3 V1V2 breadth score was inversely correlated with CRF01_AE 169K HIV-1 infection risk (OR = 0.63 per 1-SD increase, \( P = 0.023 \)) but not differentially correlated between the CC and CT/TT vaccine recipients (OR = 0.66 vs. 0.29 per 1-SD increase in CC vs. CT/TT) (Supplemental Table 7).

Furthermore, the previous immune correlates analysis (2) reported that IgG3 Env response rate but not magnitude of response to the vaccine strain A244 gp120 weakly correlated with a decreased risk of overall HIV-1 infection. We found that the magnitude of IgG3 Env response was an inverse CoR only in the CT/TT vaccine recipients, particularly with the risk of CRF01_AE 169K HIV-1 infection (OR = 0.97 vs. 0.28 per 1-SD increase in CC vs. CT/TT) (Table 3). Similarly, the magnitude of IgG3 Env response to the circulating strain AE703357 was an inverse CoR of CRF01_AE 169K HIV-1 infection only in the CT/TT vaccine recipients (OR = 0.91 vs. 0.37 per 1-SD increase in CC vs. CT/TT).
Although inverse correlations (estimated ORs < 1) were observed in the CT/TT vaccine recipients for several immune response variables, there was low statistical power for detecting significant effect modification compared with that in CC vaccine recipients, mainly due to the high estimated VE in CT/TT subjects: 7 of 41 vaccine HIV-1 cases (17%) and 2 of 25 vaccine CRF01_AE 169K HIV-1 cases (8%) carried the CT/TT genotype compared with 49 of 205 vaccine controls (24%). Therefore, while the small number of CRF01_AE 169K HIV-1 cases limits the precision of the assessment of how the CoRs depend on FCGR2C 126C>T genotype, it also makes the assessment more potentially clinically meaningful, given the connection of the inferences to the high estimated VE.

To further describe the nature of the inverse correlations in CT/TT vaccine recipients, we plotted and compared the distributions of immune responses for the 2 CT/TT CRF01_AE 169K HIV-1-infected vaccine cases and the 49 CT/TT uninfected vaccine controls and for comparison also did this for the 23 CC CRF01_AE 169K HIV-1-infected vaccine cases and the 154 CC uninfected vaccine controls (Figures 4 and 5). These 2 CT/TT cases had unusually low immune responses uniformly for all responses measured, suggesting that these 2 individuals may be interpreted as having “complete lack of take” to the vaccine. As Figures 4 and 5 illustrate, many of the immune responses were significantly higher in the uninfected CC vaccine recipients compared with those in the 23 CC CRF01_AE 169K vaccine cases. These significant results are consistent with the earlier results based on logistic regression models.

Discussion

The results of the RV144 ALVAC/AIDSVAX clinical trial suggested the contribution of Fc-mediated Ab function to the reduction of the risk of HIV-1 infection (2, 5, 23, 28). The binding of the Ab Fc region to FcRs expressed on monocytes, macrophages, neutrophils, NK cells, and dendritic cells triggers a variety of immune functions mediated by these cellular populations (6). All human FcγRs except FcγRIIB signal through an immunoreceptor tyrosine-based activating motif (ITAM), while FcγRIIB delivers inhibitory signals through an immunoreceptor tyrosine-based inhibitory motif. The FCG2RC is peculiar in its structure and is generated by an unequal crossover between FCG2RA and FCG2RB (29, 30). FCG2RC shares the extracellular sequence of FCG2RB but signals through the ITAM like FCG2RA. Interestingly, FCG2RC has been reported capable of mediating ADCC (30–32) and may play a role in anti-HIV-1 Ab–neutralizing activity, similar to FCG2RB (33).

In this study, we identified a tag SNP in FCG2RC (FCG2RC 126C>T, rs114945036) that significantly modified VE to prevent infection with CRF01_AE circulating strains in Thailand that expressed lysine at position 169 (169K). Estimated VE was 91% against CRF01_AE 169K HIV-1 and 64% against any HIV-1 strain in CT/TT subjects, in contrast to 15% and 11% in CC subjects, respectively. This SNP locates in intron 2 of FCG2RC and is in a complete LD ($r^2 = 0.9, D' = 1$) with 2 other SNPs in FCG2RC, rs138747765 in exon 3 (missense mutation) and rs78603008 in intron 3, and is in a nearly complete linkage ($r^2 = 1, D' = 1$) with another
FCGR2C SNP, rs373013207 in intron 5. The allele frequency of the minor T allele of FCGR2C 126C>T in the Thai study population was 13.5%, and the prevalence of the CT/TT genotype was 24%. Given that the estimated VE was 31% in all individuals and 64% in CT/TT individuals, the 24% prevalence of CT/TT implies that about half of the estimated 31% efficacy may be explained by the CT/TT genotype subgroup (0.24 × 0.64 = 0.15 = 0.31/2).

The same results were observed for the 2 linked SNPs in this Thai study population. The minor allele frequency of FCGR2C 126C>T (each of the 2 linked SNPs) in other populations ranges from 10% to 30% (0% to 39%) (data from the 1000 Genomes Project; ref. 34), resulting in population frequencies of individuals carrying at least one minor allele in their genotype of 21% to 53% (0% to 64%). The minor allele frequencies and prevalences of individuals carrying at least one minor allele at the FCGR2C 126C>T locus and at 2 linked SNP loci in different ethnic populations are tabulated in Supplemental Table 8.

To our knowledge, this is the first report of an association between FCGR2C 126C>T (the SNPs in linkage) and intervention efficacy against a clinical outcome. Whether this association with vaccine prevention of HIV-1 infection and the corresponding estimation of VEs are generalizable to other host populations, circulating HIV-1 strains, or other vaccines needs to be determined in new VE trials.

The functional significance of this association remains to be established. SNP rs10917661 alters a codon at amino acid position 33 in exon 3 of FCGR2C, resulting in either an open frame (FCGR2C-ORF) or a stop codon (FCGR2C-Stop), presumably affecting the functional expression of the gene (24, 30). This SNP locates between FCGR2C 126C>T and the missense SNP rs138747765 in exon 3. Since, in this study, all except one of the samples were homozygous for the FCGR2C-Stop genotype, expression of the missense mutation would not occur in this population. At this stage, the positions of the significantly associated SNPs within FCGR2C suggest at least two possibilities regarding functional associations. One possibility is that these detected SNPs for association were in linkage with functional variants that were not genotyped in this study (35).

However, the complete LD among the intron 2, exon 3, and intron 3 SNPs does suggest the second possibility of alternative splicing, yielding an open reading frame that bypasses the FCGR2C-Stop codon to encode a product with an atypical FcR protein sequence. Alternative splicing variants of FCGR2A and FCGR2B have been characterized, and, while none have been described for FCGR2C, only a limited examination of this gene has been completed to date (30). While neither the intron 2 nor intron 3 SNPs alter known splicing signals, the complete LD among the significant FCGR2C SNPs suggests that an examination of expression patterns in different cell types is warranted. In that regard, in addition to NK cells, FCGR2C transcripts have been identified in other hematopoietic compartments, including B lymphocytes and myelomonocytic cells (36).

If associated changes in mRNA expression are detected, experiments can then be designed to measure altered protein expression and consequent FcR function. Altered splicing may result in transcripts encoding novel proteins, directing the focus of future studies to establishing their expression. Functional analysis will include biochemical and cellular measurements of altered function, such as changes in Fc-binding affinity. A second and not mutually exclusive consequence of the linked variation is the quantitative differences in mRNA expression, resulting in changes in native protein expression levels. Such changes could affect overall levels of Fc uptake and consequently affect the regulation of any number of the afferent and efferent phases of the IgG-dependent immune responses (37). Due to limitations in the availability of study samples for RV144, we were not able to examine expression directly and do plan to address possible altered expression patterns associated with these genotypes in other population samples as they become available in future studies.

In RV144 vaccine recipients, IgG- and IgG3-binding Abs to V1V2 scaffold proteins inversely correlated with HIV-1 infection risk (2–4, 26, 38) and plasma IgA-binding Abs to Env directly correlated with HIV-1 infection risk (2). We found a trend for the IgA direct correlation to occur only in the CC vaccine recipients but not in the CT/TT vaccine recipients. We also found the inverse correlations of IgG- and IgG3-binding Abs to V1V2 presented in both genotype groups, but with a trend toward stronger inverse correlations in the CT/TT vaccine recipients, especially for correlation with CRF01_AE 169K-specific HIV-1 infection risk. In addition, we found several new results about inverse correlates of CRF01_AE 169K HIV-1 infection risk that were restricted to the CT/TT vaccine recipients with borderline significant interaction P values: IgG avidity, NAb to CRF01_AE circulating strains, and IgG and IgG3 Env binding to the A244 vaccine strain and to the CRF01_AE circulating strain AE703357. These inverse correlations, together with the result that the vaccine may have only (or better) protected CT/TT subjects against CRF01_AE 169K HIV-1, generate a hypothesis that these Ab responses are correlates of protection only in (or more strongly in) individuals carrying the CT or TT genotype. Env-specific IgA Abs may block protective IgG Fc-mediated effector functions to the recipients who carry the CC genotype. Because correlates of risk analyses do not establish correlates of protection nor mechanisms of protection (39), additional research would be needed to validate or refute these Ab responses as FCGR2C genotype–dependent correlates of protection.

Of particular note, the 2 CRF01_AE 169K HIV-1–infected vaccine recipient cases in the CT/TT genotype group had uniformly low immune responses of all types (Figures 3 and 5 and Supplemental Figures 2–4). While definite conclusions from these 2 cases cannot be drawn, the data may provide a clue that the CT/TT group with “vaccine take” could have received a relatively high degree of protection against CRF01_AE 169K HIV-1. Historically, in VE trials, correlates of protection have been documented in part by observing that breakthrough vaccine recipient cases have unusually low immune responses (40). An alternative explanation is that the measured immune responses are not correlates of protection, but the CT/TT genotype is associated with protection through an unmeasured immune mechanism. A third possible explanation is a false-positive correlate of risk in CT/TT subjects due to statistical sampling variability. Additional research would be needed to discriminate among these possible underlying reasons for the observed association. Overall, it will be important to study the impact of different subclasses of Fc-mediated IgG functions; for instance, to evaluate IgG1 and IgG3 for their ability to
mediate ADCC, phagocytosis, and favor neutralization by recruiting FCGR2C-bearing cells in a group of individuals with the CT/TT genotype. It will be crucial to understand how these cells will respond following FCGR engagement compared with cells that do not express the FCGR2C. This will help explain the interplay between the adaptive humoral response and the innate immune response in conferring vaccine-induced protection.

None of the immune responses studied significantly differed between CT/TT and CC vaccine recipients (Figure 3 and Supplemental Figures 2–4). This result combined with the results on effect modification of correlates of risk suggest that any role of the FCGR2C 126C>T genotype to modify VE cannot be explained by greater levels of immune response in the CT/TT subgroup, but rather by a different quality or function of immune response in CT/TT vaccine recipients compared with CC vaccine recipients. Since mechanistic correlates of protection remain elusive for most vaccines (39, 41, 42), the characterization of host genetics as it interacts with vaccine-induced immunity allows new biology on FcR-mediated immunity to be tested. For HIV-1 infection, profound insights on HIV-1 control and disease progression have come from analyses of host genetics, viral genetics, and host immunity (43, 44). Our study now demonstrates a role for FcR host genetics and HIV-1 vaccine-induced immunity.

Taken together, our findings show the importance of considering host immune genetics in the assessment of VE, immune correlates, and vaccine response trajectories between the CC and CT/TT vaccine groups to assess whether responses were more durable in the CT/TT group and to assess whether the polymorphism modifies the association of Abs over time with infection risk. In addition, to help understand the generalizable impact of these observations, surveys of the polymorphisms may be conducted in global populations relevant to HIV-1 vaccine design, including in Thailand, southern Africa, East and West Africa, the Americas, Europe, and Australia. It also may be informative to measure FcR genetics in populations in which nonfocal vaccines were identified, in particular for the Merck Ad5 vaccine tested in the HVTN 502 Step trial (45, 46) and the VRC vaccine tested in the HVTN 505 trial (47), each conducted predominantly in men of mixed European descent who have sex with men in the Americas. A lower prevalence of CT/TT in this population compared with the RV144 population would provide a potential partial explanation for lack of efficacy, although this may be unlikely given that CT/TT is more prevalent in people of mixed European descent than in people from Thailand. The results also suggest elevating FCGR2C genotyping and functional studies to secondary analyses in upcoming, scientifically intensive phase 1/II HIV vaccine trials. Last, these results could affect vaccine design if and when future research identifies potential functional mechanisms of CT/TT-dependent RV144 vaccine protection, which would motivate development of new candidate vaccines that can extend the protective mechanism to CC individuals.

Methods

Study cohorts. The case cohort, consisting of all 125 subjects who acquired HIV-1 infection during the RV144 trial (74 placebo and 51 vaccine recipients), was used to assess effect modification of VE by the FcR genotypes. The case-control cohort, consisting of the 41 infected and 205 uninfected vaccine recipients with week 26 immune responses measured, was used to assess effect modification of CoRs by the FcR genotypes. These 205 uninfected vaccine recipients were used for comparing immune responses between vaccinated subgroups with different FcR genotypes. The 205 controls were selected previously in the initial immune correlates study, using a stratified random sample of vaccine recipients free of HIV-1 infection at 42 months, with strata defined by the cross-classification of gender, the number of vaccinations received, and per-protocol status (2).

FcR genotyping. Target amplicons for resequencing were generated by defining PCR primers from the genomic templates spanning each of the 5 FCGR loci on chromosome 1q23-24 and the FCAR locus on chromosome 19q13.42, using reference human genome build 37. The external protein domains and the transmembrane sequences of the 5 FCGR loci and the complete coding sequence of FCAR locus were targeted (Supplemental Figure 1). Amplicon resequencing was carried out using dye terminator chemistry, and data were analyzed with software as described previously (48). The primer sequences and associated information regarding resequencing are available in Supplemental Table 1. For more details, see the Supplemental Methods.

Immune response assays. We studied the 6 primary immune response variables assessed in the original immune correlates study (2), all measured 2 weeks after the last immunization at the week 26 visit: plasma IgA-binding Ab to Env, IgG-binding Ab to gp70-V1V2, avidity of IgG Ab to Env, ADCC, NAbs, and the level of Env-specific CD4 T cells. We also studied the 8 “sensitivity variables” assessed in the same study (2) that were related to the 6 primary variables within the same assay types as well as the 1 secondary variable in the study that was a significant CoR, with a false discovery rate q value below 0.20 (IgA C1 peptide). We also studied IgG and IgG3 responses to the vaccine strain gp120 (A244) and to the CRF01_AE circulating strain AE703357, which were studied as secondary variables in ref. 2. These 2 HIV-1 Env antigens are hypothesized to be relevant Env target sequences for effective FcR-mediated vaccine-elicited immunity in RV144 trial. In addition, we studied immune response variables that were evaluated as CoRs in a later study (4): IgG Abs to 11 V1V2 scaffold antigens, measured using an ELISA and a binding Ab multiplex assay (BAMA). Last, we studied IgG3 Abs to the same 11 V1V2 scaffold antigens studied previously (26). Because ELISA and BAMA yielded very similar CoR results for the IgG and IgG3 Abs to V1V2 scaffold antigens and Env antigens, we only presented the BAMA results for these variables. Supplemental Figures 5 and 6 show heat maps and hierarchical clustering trees of Spearman rank correlations of pairs of the IgG and IgG3 Abs to the 11 V1V2 scaffold antigens.

Statistics. The case-only method, which uses data from infected subjects only, was used to estimate overall and HIV-1 genotype–specific VE stratified by FcR SNP genotypes and to test for SNP effects, wherein VE differs among FcR SNP genotype subgroups (21). The case-only method is valid only if the trial is randomized and has a rare study outcome, and the RV144 trial meets both criteria. This case-only method is optimally powerful (adding SNP data from uninfected subjects would not increase statistical power) (49, 50) and cost-effective.
because only data from infected cases are needed. Given the moderate sample size and the moderate minor allele frequencies for most SNPs, the SNP effects were tested under a dominant model of inheritance. Given that RV144 was a double-blind, randomized HIV vaccine trial that was conducted in Thailand alone, the host genetic factors are expected to be equally distributed between the vaccine and placebo groups and the study population was relatively genetically homogeneous. Therefore, no adjustment for subpopulation structure is needed in the statistical analysis. P values were adjusted to control the FWER using Westfall and Young’s resampling method (51), which is a more efficient method of adjusting correlated P values (resulting from correlated SNPs genotypes) than the Bonferroni adjustment method. An adjusted P value of less than 0.05 was considered significant. The sieve analysis for assessing differential VE within host genotype groups and for comparison between genotype groups used the method described by Dai et al. (21).

Logistic regression models accounting for the immune response sampling design were used to assess whether and how FcR SNP genotypes modified the correlation between week 26 immune responses and HIV-1 infection risk in vaccine recipients. Two infection risks were assessed: overall risk from any HIV-1 genotype and specific risk for CRF01_AE 169K HIV-1. In the first analysis, 41 cases and 205 controls were used, while in the second analysis, 25 CRF01_AE 169K HIV-1 cases and 205 controls were used. The fitting method accommodates the outcome-dependent stratified immune response biomarker sampling design via maximum likelihood estimation (52). All regression analyses were adjusted for gender and baseline risk behavior, as done in the initial immune correlates study (2). An interaction term of immune response variable and SNP genotype was included in models to test whether immune response differentially correlated with infection risk according to SNP genotype. The immune responses were modeled as quantitative variables (magnitudes) and as binary variables. Binary variables included high versus medium/low immune responses for tertile variables (tertiles defined by lower, middle, and upper third of response values) and positive versus negative immune responses for response variables, as defined previously (2, 4, 26). False discovery rate (q value) multiplicity correction (53) was performed separately for the set of 6 primary variables and for the set of 8 sensitivity variables and 1 secondary variable, with significance threshold q ≤ 0.20. No multiplicity correction was performed for IgG or IgG3 responses to the 11 V1V2 scaffold proteins or the 2 Env-specific antigens, because the variables belonged to the same assay type and were highly correlated with one another. A P value of less than 0.05 was considered significant.

Box plots were used to describe the distribution of each immune response variable, which was stratified by FcR SNP genotype, for uninfected vaccine recipients and the 2 infected case groups. Distributions of immune responses were compared within SNP genotype groups among the uninfected vaccine recipient controls, with Wilcoxon rank-sum and Fisher’s exact tests. Distributions of immune responses were also compared within SNP genotype groups among the vaccine recipients who were uninfected from any HIV-1 strains and those infected from CRF01_AE 169K HIV-1. For the comparative analyses, we report the Wilcoxon rank-sum test results if the subgroup-pooled response rate exceeded 20% and the Fisher’s exact test resulted otherwise.

Hierarchical clustering trees, based on a distance measure defined as 1 minus the Spearman rank correlation coefficient, and all vaccine recipients were used to cluster antigens into common antigenic groups. Study approval. The study protocol was reviewed and approved by the ethics committees of the Ministry of Public Health, the Royal Thai Army, and Mahidol University and the Human Subjects Research Review Board of the US Army Medical Research and Material Command, Fort Detrick, Maryland, USA. It was also independently reviewed and endorsed by the World Health Organization and the Joint United Nations Program on HIV/AIDS and by the AIDS Vaccine Research Working Group of the National Institute of Allergy and Infectious Diseases (NIAID) at the NIH. Written informed consent was obtained from all volunteers, who were required to pass a written test of understanding.

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