Isotype-switched immunoglobulin G antibodies to HIV Gag proteins may provide alternative or additional immune responses to 'protective' human leukocyte antigen-B alleles in HIV controllers

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Background: Natural control of HIV infection is associated with CD8$^+$ T-cell responses to *Gag*-encoded antigens of the HIV core and carriage of 'protective' human leukocyte antigen (HLA)-B alleles, but some HIV controllers do not possess these attributes. As slower HIV disease progression is associated with high levels of antibodies to HIV Gag proteins, we have examined antibodies to HIV proteins in controllers with and without 'protective' HLA-B alleles.

Methods: Plasma from 32 HIV controllers and 21 noncontrollers was examined for immunoglobulin G1 (IgG1) and IgG2 antibodies to HIV proteins in virus lysates by western blot assay and to recombinant (r) p55 and gp140 by ELISA. Natural killer (NK) cell-activating antibodies and FcγRIIa-binding immune complexes were also assessed.

Results: Plasma levels of IgG1 antibodies to HIV Gag (p18, p24, rp55) and *Pol*-encoded (p32, p51, p66) proteins were higher in HIV controllers. In contrast, IgG1 antibodies to Env proteins were less discriminatory, with only antigp120 levels being higher in controllers. High-level IgG2 antibodies to any Gag protein were most common in HIV controllers not carrying a 'protective' HLA-B allele, particularly HLA-B*57 ($P = 0.016$). HIV controllers without 'protective' HLA-B alleles also had higher plasma levels of IgG1 antip32 ($P = 0.04$). NK cell-activating antibodies to gp140 Env protein were higher in elite controllers but did not differentiate HIV controllers with or without 'protective' HLA-B alleles. IgG1 was increased in FcγRIIa-binding immune complexes from noncontrollers.

Conclusion: We hypothesize that isotype-switched (IgG2+) antibodies to HIV Gag proteins and possibly IgG1 antip32 may provide alternative or additional immune control mechanisms to HLA-restricted CD8$^+$ T-cell responses in HIV controllers.

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Keywords: antibody-dependent cell-mediated cytotoxicity, controllers, HIV, immune complexes, immunoglobulin G2 antibodies

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Introduction

Understanding how the immune system of HIV controllers contains the infection may facilitate the development of therapeutic and possibly preventive HIV vaccines. Natural control of HIV infection is associated with $CD8^+$ T-cell responses to HIV core antigens encoded by *Gag* [1,2] and carriage of particular human leukocyte antigen (HLA)-B alleles, especially HLA-B*57 but also HLA-B*27, -B*14 and -B*52 and possibly others [3–5]. However, some HIV controllers do not carry these ‘protective’ HLA-B alleles [3,4], suggesting that immune responses other than $CD8^+$ T-cell responses contribute to control of HIV infection. Genetic studies implicate natural killer (NK) cells [6], and there is an increasing interest in antibody-dependent cell-mediated cytotoxicity (ADCC) [7,8].

Numerous studies [9–25] have demonstrated that progression of HIV disease is slower in adults and children with higher serum levels and/or avidity of immunoglobulin G (IgG) antibodies to HIV Gag proteins (p17, p24, p55). Although these antibodies may be markers of $CD4^+$ T-cell help [26], they might have a direct role in the control of HIV replication, particularly antibodies of the IgG2 subclass. Ngo-Giang-Huong *et al.* [27] examined the relationship of plasma levels of IgG1 and IgG2 antibodies to HIV proteins with rates of disease progression and plasma HIV RNA levels in 71 HIV patients who were initially classified as long-term nonprogressors. Whereas IgG2 antibodies to gp41 (possibly reflecting an IgG2 antibody response to multiple HIV proteins) were associated with slower disease progression, lower plasma HIV RNA levels were associated with IgG2 antibodies to p55 and p24. Also, we have demonstrated that vaccination of HIV patients receiving antiretroviral therapy (ART) with a recombinant DNA vaccine encoding a fowlpox virus vector, HIV *Gag-Pol* and interferon-gamma (IFN-γ) increased IgG antibodies to vaccine antigens, including IgG2 antibodies to p24, which were associated with partial control of HIV replication after ART was ceased. This was particularly so in individuals carrying a genetic polymorphism of Fcγ receptor (R) IIa that confers a higher affinity of IgG2 binding to FcγRIIa [28]. However, Banerjee *et al.* [29] demonstrated that plasma levels of IgG2 antibodies to HIV Gag antigens did not differ between HIV controllers and ‘chronic progressors’, though they did show that plasma levels of IgG1 antibodies to HIV gp120 and p24 were higher in HIV controllers. A notable difference between these studies was the use of recombinant HIV antigens in ELISAs by Banerjee *et al.* [29] and viral lysates in denaturing western blot assays in the other studies. It is therefore possible that antigen conformation affects the detection of IgG2 antibodies to HIV antigens.

Isotype diversification of IgG antibodies occurs through class switch recombination of immunoglobulin heavy chain genes, with switching to IgG2 occurring downstream of IgG3 and IgG1 [30], and results in broadening of antibody function. IgG2 antibodies facilitate phagocytosis of antigens through covalent dimerization [31] and preferential binding to FcγRIIa [32,33], which primarily mediates phagocytosis of antibodies bound to antigens [34]. IgG2 antibodies and FcγRIIa play important roles in the phagocytosis of encapsulated bacteria by neutrophils [35], but FcγRIIa is also the most abundant FcγR on plasmacytoid dendritic cells (pDCs) [36,37], suggesting that FcγRIIa, and possibly IgG2 antibodies, might affect antiviral immune responses mediated by pDCs [38]. Furthermore, IgG2 is the predominant IgG subclass in circulating immune complexes of healthy individuals [39], suggesting that IgG2 antibodies influence the phagocytosis of immune complexes. Of note, HIV infection attenuates IgG2 antibody responses [40].

Here, we have assessed IgG1 and IgG2 antibodies to HIV proteins using western blot assays and ELISAs as well as examined NK cell-activating antibodies, as a surrogate of ADCC activity, and FcγRIIa-binding immune complexes in plasma samples from HIV controllers and noncontrollers. Comparison of antibody responses was undertaken in HIV controllers who did or did not carry ‘protective’ HLA-B alleles.

Materials and methods

Patients

Cryopreserved plasma was obtained from 32 HIV controllers who had a plasma HIV RNA level of less than 2000 copies/ml [of whom 14 (44%) were elite controllers with levels of <50 copies/ml] on at least three occasions over at least 12 months without ART [4] and 21 HIV noncontrollers who had a plasma HIV RNA level of more than 10 000 copies/ml, a $CD4^+$ T-cell count of less than 100/μl and had not received ART. All patients had provided informed consent for the study.

Western blot assay for immunoglobulin G1 and immunoglobulin G2 antibodies to HIV proteins

IgG1 and IgG2 antibodies to HIV proteins from viral lysates were assayed by western blot on the basis of the method described previously [41] using biotinylated mouse antihuman IgG1 (Sigma, #B6775; Sigma-Aldrich Pty Ltd, Castle Hill, Australia) or IgG2 (Invitrogen, #05–3540; Invitrogen Corporation, Camarillo, California, USA) at 1 : 1000 dilution and alkaline phosphatase-conjugated streptavidin (Invitrogen #SSN1005) at 1 : 30 000 dilution. Band intensities were scored from 0 to 4.

ELISA for antibodies to gp140 Env protein

IgG1 and IgG2 antibodies to gp140 Env protein were assayed by an ELISA [42], adapted to detect IgG subclasses, in a half-log dilution series using gp140 derived from the subtype B R5-tropic HIV-1_{AD8} strain and horse radish peroxidase (HRP)-conjugated mAbs to human IgG1 or IgG2 (Invitrogen; clones HP6069 and HP6014, respectively). Wells were considered positive when optical density (OD) was at least three-fold higher (IgG1) or two-fold higher (IgG2) than the OD obtained with HIV-negative human sera.

ELISA for antibodies to Gag

HIV-1_{IIIB} p55 Gag (NIH AIDS Research and Reference Reagent Program, catalogue #3276) was supplemented with 1% SDS and incubated at 37°C for 30 min to improve solubility prior to absorbing onto ELISA plates (200 ng/well) in coating buffer (20 mmol/l Tris pH 8.8, 100 mmol/l NaCl) overnight at room temperature. Wells were blocked with 5% skim milk powder in PBS/0.1% Tween 20 for 1 h followed by addition of plasma samples diluted 100-fold in blocking buffer. After 4 h incubation and washing with PBS/0.1% Tween 20, HRP-conjugated antibodies to human IgG1 or IgG2 in blocking buffer were added and incubated for 1 h. After washing, ELISAs were developed using standard techniques. Background was defined using HIV-negative human sera. Samples were considered positive when the OD was at least two-fold higher than background.

Natural killer cell activating antibodies

Antibody-induced cytokine production in NK cells was assessed as a surrogate of ADCC, as described previously [8,43]. Briefly, 150 μl of healthy donor whole blood and immunoglobulin purified from 50 μl of patient plasma was incubated at 37°C with either overlapping 15-mer HIV-1 peptide pools spanning consensus subtype B Gag or Env (NIH AIDS Reagent Repository) or gp140 Env protein (1 μg/ml) for 5 h in the presence of Brefeldin A and monensin (10 μg/ml, Sigma). Following incubation, $CD3^-$ $CD2^+$ $CD56^+$ lymphocytes were analysed for expression of intracellular IFNγ. Fluorescent antibodies used were CD3 (catalogue number 347344, fluorescent label PerCP), CD2 [556611, fluorescein isothiocyanate (FITC)], CD56 (555516, PE) and IFNγ (557995, Alexa700) (all from BD Biosciences, North Ryde, Australia). We also studied killing of gp140-pulsed CEM.NKr cells in the rapid fluorescent ADCC (RFADCC) assay as previously described [44].

Analysis of immunoglobulin G1+ and immunoglobulin G2+ FcγRIIa-binding immune complexes

Plasma was thawed at 37°C and centrifuged (300*g*, 3 min) to remove aggregates. Immune complexes were precipitated from plasma by incubation with 3.5% polyethylene glycol 6000 dissolved 1 : 40 in 0.1 mol/l borate (pH 8.4) at 4°C for 16 h. Following centrifugation (2500*g*, 15 min), supernatants (containing uncomplexed antibody) were discarded. Pellets (containing immune complexes) were resuspended in PBS and incubated at 37°C until dissolved. Immune complexes were incubated with 5×10^5 IIA1.6 cells at 4°C for 20 min in duplicate. IIA1.6 is a mouse B lymphoma cell line that has been transfected with human FcγRIIa (donated by Professor Mark Hogarth). IIA1.6 cells were kept on ice at all times to prevent internalization of surface receptors, and expression of FcγRIIa was confirmed in each assay using a mouse antibody to FcγRIIa (Mab8.7, donated by Professor Mark Hogarth). Cells incubated with immune complexes were washed twice in PBS (300*g*, 2 min), resuspended and stained with either antihuman IgG1-FITC or antihuman IgG2-FITC (Sigma Life Sciences, clones 8c/6-39 and HP-6014, respectively) diluted 1 : 1000 with PBS (4°C, 20 min). Cells were washed twice in PBS (300*g*, 2 min), resuspended in 1% bovine serum albumin/PBS and acquired on a FACS Canto II flow cytometer. Data were analysed using FlowJo software (TreeStar Inc., Ashland, Oregon, USA) and results expressed as mean fluorescence intensity of the cell histogram.

Human leukocyte antigen-B typing

HLA typing was undertaken by sequenced-based typing using genomic DNA in the Department of Clinical Immunology, Royal Perth Hospital or Red Cross Blood Service, Sydney. Both laboratories are accredited by the American Society for Histocompatibility and Immunogenetics (ASHI).

Statistics

Plasma levels of antibodies and immune complexes and ADCC activity were compared using Mann–Whitney *U* tests. The frequency of high-level antibodies in groups of patients was compared by Chi-squared tests.

Results

Immunoglobulin G1 antibodies to HIV Gag and *Pol*-encoded proteins were higher in HIV controllers

We examined plasma samples for IgG1 and IgG2 antibodies to HIV Gag (p18, p24), *Pol*-encoded (p32, p51, p66) and Env (gp41, gp120) proteins in virus lysates using western blot assays and to recombinant Gag (rp55) and Env (rgp140) proteins using ELISAs (Fig. 1). Plasma levels of IgG1 antibodies to all HIV proteins detected by western blot assay were higher in HIV controllers than noncontrollers, except for antigp41, which were high (western blot band score of 3 or 4) in all patients (Fig. 1a). Plasma samples were tested for IgG1 antirp55 by ELISA at a single dilution on two occasions with very good concordance between assays. Twenty-six of 32 (81%) HIV controllers had a positive IgG1 antibody to rp55 compared with five of 10 (50%) noncontrollers ($P = 0.09$) (Fig. 1c). The titre of IgG1 antibody to rgp140 was also determined by ELISA and endpoint titres were not

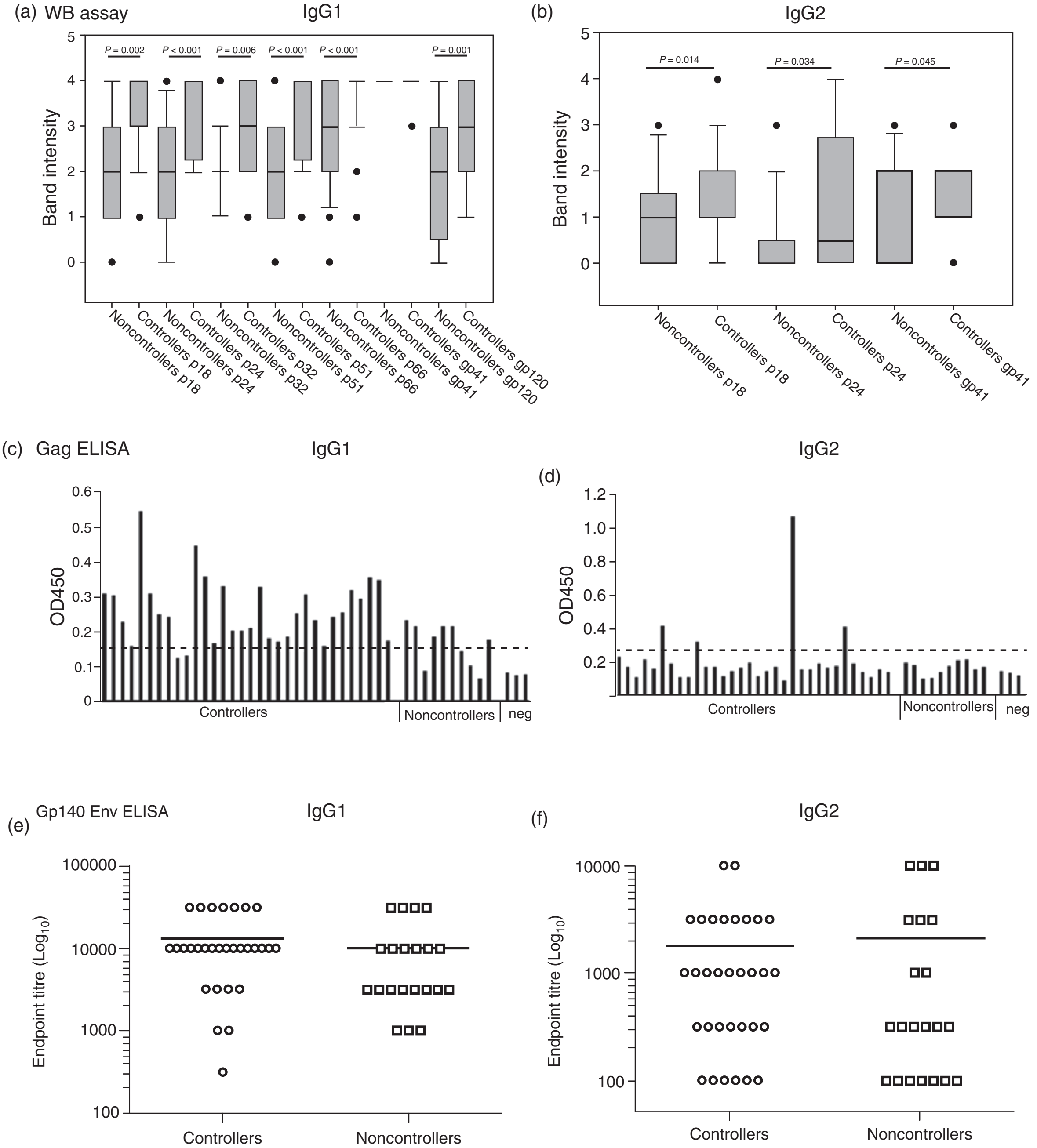

Fig. 1. IgG1 and IgG2 antibodies to HIV proteins from a virus lysate assayed by western blot assay (a, b) and to Gag (rp55) (c, d) or rgp140 Env protein (e, f) assayed by ELISA in HIV controllers and noncontrollers. Western blot assay band intensities were scored from 0 to 4. Gag ELISA results are reported as optical densities with the dashed line indicating the cut-off for a positive result. Antibodies to rgp140 Env protein are reported as endpoint titres.

significantly different between HIV controllers and noncontrollers ($P = 0.12$) (Fig. 1e).

Immunoglobulin G2 antibodies to HIV Gag proteins were most common in HIV controllers

By western blot assay, IgG2 antibodies to gp120 and *Pol*-encoded proteins were not detected. IgG2 antibodies to p18, p24 and gp41 produced more intense bands in HIV controllers than noncontrollers, but, notably, the differences were more marked for antip18 and antip24 than for antigp41 (Fig. 1b). Plasma samples were also tested for IgG2 antibodies to rp55 and rgp140 by ELISA as indicated above for IgG1 antibodies. Four of 32 (12.5%) HIV controllers had a positive IgG2 antibody to rp55 compared with none of 10 noncontrollers ($P = 0.14$) (Fig. 1d). Of note, only one HIV controller with positive IgG2 antibodies to rp55 had high-level IgG2 antibodies to Gag proteins detected by western blot

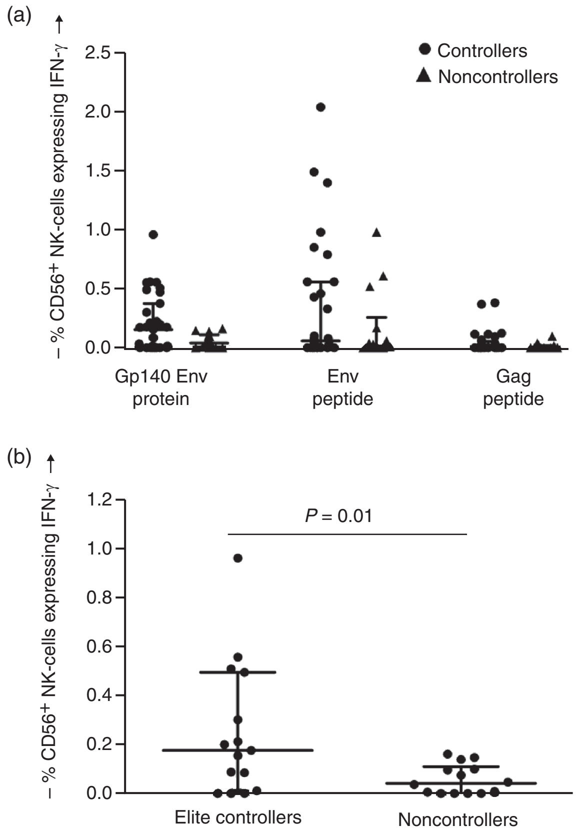

Fig. 2. Natural killer cell activating antibody activity with gp140 Env protein, Env peptide pool and Gag peptide pool in plasma from HIV controllers and noncontrollers (a). As there was a trend towards higher values for gp140 Env protein in HIV controllers, elite controllers were compared separately with noncontrollers (b). Values are reported as the percentage of CD56$^+$ lymphocytes expressing IFN-γ after incubation with immunoglobulin samples. IFN-γ, interferon-gamma.

assay. The titre of IgG2 antibody to gp140 Env protein did not differ between HIV controllers and noncontrollers ($P = 0.34$; Fig. 1f).

Natural killer cell activating antibodies to HIV rgp140 Env protein were higher in HIV controllers

NK cell activating antibodies to pools of HIV Env and Gag peptides and to rgp140 Env protein were assessed by flow cytometry [8,43]. Antibody-dependent NK cell activity against Gag peptides was generally low and activity against Env peptides was not significantly different between HIV controllers and noncontrollers ($n = 14$) (Fig. 2a). However, activity against rgp140 Env protein was marginally higher in HIV controllers than noncontrollers ($P = 0.09$) (Fig. 2a). We therefore compared elite controllers with noncontrollers and found significantly higher activity against rgp140 Env protein in elite controllers ($P = 0.01$, Fig. 2b). Activity against rgp140 Env protein in the RFADCC assay [44] was not different between controllers and noncontrollers (data not shown).

Immunoglobulin G2 antibodies to Gag proteins and immunoglobulin G1 antip32 differentiated HIV controllers without ‘protective’ human leukocyte antigen-B alleles from those with these alleles

If antibodies to HIV proteins contribute to control of HIV replication, they are likely to be higher in HIV controllers who do not carry ‘protective’ HLA-B alleles. Twenty of the 32 (62.5%) HIV controllers carried a ‘protective’ HLA-B allele (B*57, B*52, B*27 or B*14+Cw0802), as defined in the International HIV Controllers Study [4]. We therefore compared antibodies in HIV controllers who did or did not carry these alleles (Table 1). Plasma levels of IgG1 antibodies to p24, p51, p66, gp41 and gp120, IgG2 antibodies to gp41 and NK cell-activating antibodies to gp140 Env protein did not differ between groups ($P > 0.42$). We then interrogated the data for IgG1 antip18 and antip32 and IgG2 antip18 and antip24 ($P < 0.16$) to determine whether there might be an interaction with HLA-B alleles. For this analysis, patients with HLA-B*57 ($n = 10$) or HLA-B*52 ($n = 2$) and HLA-B*27 (without -B*57) ($n = 9$) or B*14+Cw0802 ($n = 1$) were grouped together because the association with HIV control is stronger for the former than latter alleles [4]. In addition to the differences demonstrated between HIV controllers and noncontrollers (Fig. 1), the clearest association of an antibody response with control of HIV infection was for IgG2 antip24 in patients who did not carry a ‘protective’ HLA-B allele or who carried HLA-B*27 or B*14+Cw0802 (Fig. 3).

To investigate the possible association of IgG2 antibodies to HIV Gag proteins with control of HIV infection further, we compared patients with HLA-B*57 or HLA-B*52, with HLA-B*27 or B*14+Cw0802 or with no ‘protective’ HLA-B alleles for evidence of a high-level IgG2 antibody response to any Gag protein (western blot score of 3 or 4 for antip18 or antip24 and/or positive antirp55). Such an antibody response was demonstrated in two of 12 patients with HLA-B*57 or HLA-B*52 (16.5%), four of eight patients with HLA-B*27 or B*14+Cw0802 (50%) and nine of 12 patients without ‘protective’ HLA-B alleles (75%) ($P = 0.016$ overall and 0.004 for trend, by Chi-square test).

Immunoglobulin G1 and immunoglobulin G2 content of plasma FcγRIIa-binding immune complexes was similar to non-HIV individuals in HIV controllers but not noncontrollers

We also examined the IgG1 and IgG2 content of plasma FcγRIIa-binding immune complexes in HIV controllers and noncontrollers ($n = 10$) (Fig. 4a). Consistent with findings for IgM-IgG immune complexes purified from normal human plasma [39], FcγRIIa-binding immune

Table 1. Comparison of antibody responses to HIV proteins in HIV controllers who do or do not carry 'protective' human leukocyte antigen-B alleles.

Antibody response[a]	HIV controllers without 'protective' HLA-B alleles[b] (median, IQR)	HIV controllers with 'protective' HLA-B alleles (median, IQR)	P^c
IgG1 antip18	4 (3–4)	3 (2–4)	0.11
IgG1 antip24	4 (3–4)	4 (2–4)	0.65
IgG1 antip32	3.5 (2.25–4)	2 (2–3)	0.04
IgG1 antip51	4 (2.25–4)	4 (2.25–4)	0.65
IgG1 antip66	4 (4–4)	4 (3.25–4)	0.42
IgG1 antigp41	4 (4–4)	4 (4–4)	1.0
IgG1 antigp120	3.5 (2–4)	3 (2.25–4)	0.95
IgG2 antip18	1.5 (1–3)	1 (1–2)	0.10
IgG2 antip24	1.5 (0–3.75)	0 (0–2)	0.16
IgG2 antigp41	1 (1–2)	1 (1–2)	0.91
ADCC antibodies to gp140 Env protein	0.218 (0.024–0.412)	0.183 (0.048–0.634)	0.51

ADCC, antibody-dependent cell-mediated cytotoxicity; HLA, human leukocyte antigen; IFN-γ, interferon-gamma; IQR, interquartile range; WB, western blot.

[a] All antibody responses are reported as WB assay band scores except for ADCC antibodies to gp140 Env protein, which are reported as IFN-γ activity.

[b] Protective HLA-B alleles = HLA-B*57, HLA-B*27, HLA-B*1402+Cw0802 or HLA-B*52 [4].

[c] By Mann–Whitney test.

complexes from non-HIV individuals ($n = 12$) contained much more abundant IG2 than IgG1. Similarly, IgG2 was more abundant than IgG1 in FcγRIIa-binding immune complexes from HIV controllers. In contrast, IgG1 was as abundant as IgG2 in FcγRIIa-binding immune complexes from noncontrollers. As HIV controllers had more abundant IgG1 in FcγRIIa-binding immune complexes than non-HIV individuals, we compared elite controllers and virological controllers and found that IgG1 was more abundant in the latter group (Fig. 4b).

Discussion

We have demonstrated that particular antibody responses to HIV proteins are associated with control of HIV infection and might contribute to immune control by a mechanism that is distinct from $CD8^+$ T-cell responses restricted by 'protective' HLA-B alleles. As well as confirming that IgG2 antibodies to gp41 are associated with control of HIV infection [27], we demonstrated larger amounts of IgG2 antibodies to HIV Gag proteins in HIV controllers than noncontrollers. Moreover, a high-level IgG2 antibody response to any Gag protein (p18, p24 or rp55) correlated with control of HIV infection in patients who did not carry HLA-B*57 more strongly than any of the other antibody responses examined. Plasma levels of IgG1 antip32 were also higher in HIV controllers who did not carry 'protective' HLA-B alleles. In addition, we have confirmed previous reports that IgG1 antibodies dominate the IgG antibody response to all HIV proteins including in HIV controllers [29,45]. We interpret these findings as evidence that IgG2 antibodies to HIV Gag proteins may contribute to protective immune responses against HIV in controllers who do not carry HLA-B*57. As HLA-B*57 is the strongest correlate of immune control of HIV infection [3–5,46] and recognizes more viral epitopes than other HLA-B molecules [46], $CD8^+$ T-cell responses associated with HLA-B*57 may be sufficient in the absence of other immune responses. The finding that IgG1 antip32 were higher in HIV controllers who did not carry 'protective' HLA-B alleles was of interest because Pol may be a target of ADCC antibodies [47].

Antibody-dependent NK cell activity against HIV gp140 Env protein was higher in HIV controllers than noncontrollers, though differences were not statistically significant. However, in a posthoc analysis, we found that activity against HIV envelope protein was higher in elite controllers, consistent with the findings of Lambotte *et al.* [7]. Antibody-dependent NK cell activity against HIV envelope protein, however, did not differentiate HIV controllers with or without 'protective' HLA-B alleles.

Production of IgG2 antibodies to HIV Gag proteins in HIV controllers might be unexpected because IgG2 antibodies are thought to react preferentially with carbohydrate antigens [48], including glycosylated regions of HIV gp120 [49]. However, IgG2 antibodies are produced against protein antigens of other persistent pathogens, such as *Plasmodium falciparum*, and are associated with protection from infection [50]. It is therefore conceivable that IgG antibodies to HIV Gag proteins that have isotype switched to IgG2 are associated with control of HIV infection. Alternatively, these antibodies may be a marker of Th1 responses against Gag proteins in HIV controllers [1,2,27]. However, we have demonstrated that IgG2 antibodies to Gag proteins were most frequent in HIV controllers without 'protective' HLA-B alleles. Although IgG2 binds less avidly to FcγRIIa than IgG3 or IgG1 [32,33], this IgG

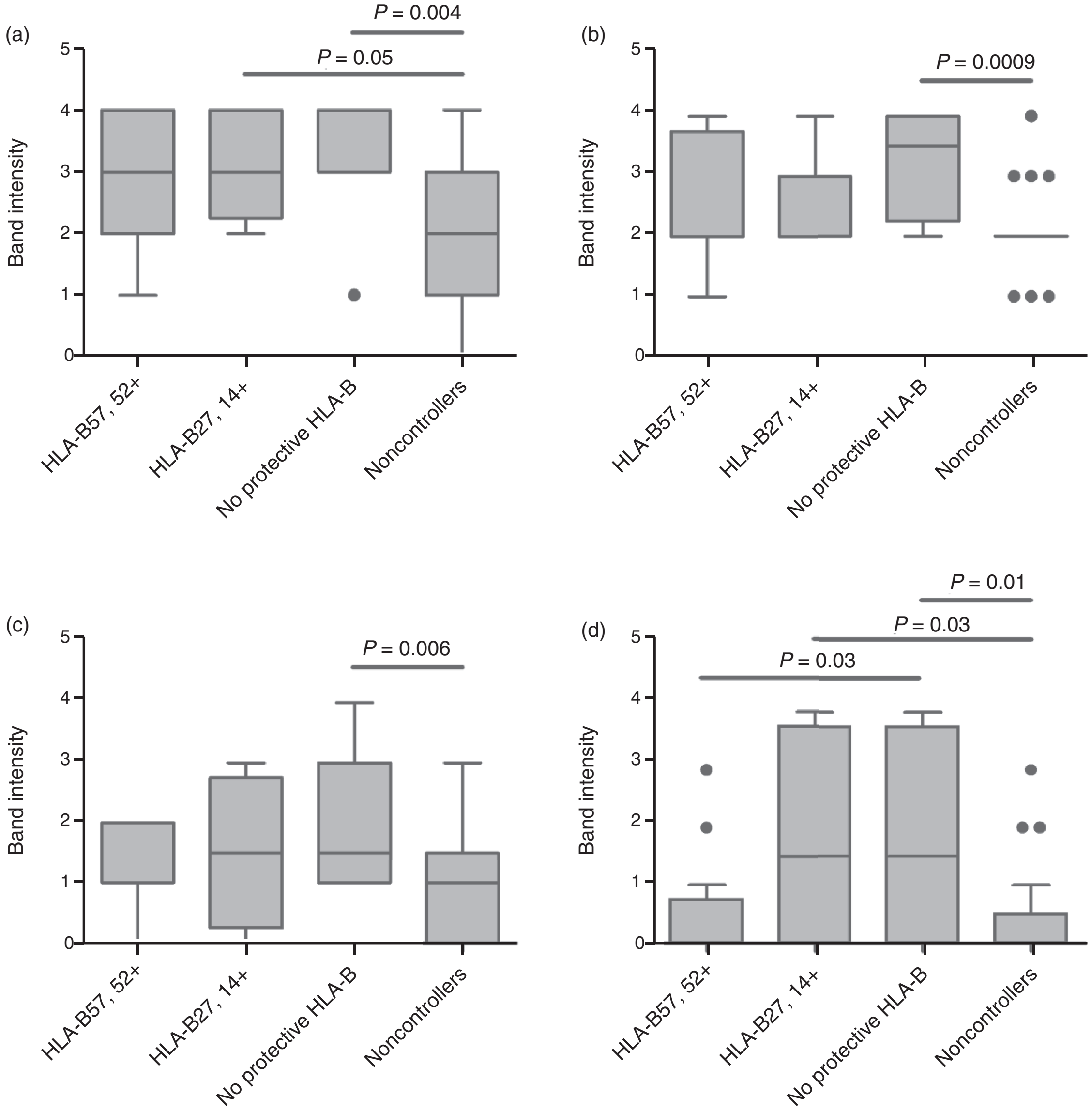

Fig. 3. Plasma levels of IgG1 antip18 (a), IgG1 antip32 (b), IgG2 antip18 (c) and IgG2 antip24 (d) in HIV controllers with HLA-B*57 or -B*52, HLA-B*27 or HLA-B*14+Cw0802 or no 'protective' HLA-B alleles. Patients carrying HLA-B*52 ($n = 2$) were grouped with those carrying HLA-B*57 ($n = 10$) and patients carrying HLA-B*14+Cw0802 ($n = 1$) were grouped with those carrying HLA-B*27 without HLA-B*57 ($n = 9$) because the association with control of HIV infection in the International HIV Controllers study [4] was stronger for HLA-B*57 or HLA-B*52 [odds ratio (OR) > 6.3] than for HLA-B*27 or HLA-B*14+Cw0802 (OR = 2.58–3.41).

subclass is distinguished from others by an ability to form covalent dimers [31] and resistance to the adverse effects of deglycosylation of the Fc region on binding to FcγRIIa [51]. HIV infection is associated with degalactosylation of the Fc region of IgG, particularly IgG1 [52], though it is unknown whether this is associated with more extensive deglycosylation that might decrease binding of IgG1 to FcγRIIa [51]. We suggest that IgG2 antibodies to HIV Gag proteins may contribute to immune control of HIV infection by broadening and enhancing the function of IgG antibodies.

Forthal *et al.* [49] demonstrated that IgG2 antibodies to gp120, elicited by vaccination with recombinant gp120, inhibited the phagocytosis of opsonized HIV-1 viral-like particles by monocytes from healthy individuals and argued that IgG2 antibodies adversely affect antiviral antibody activity. Monocytes express both FcγRI (a high-affinity FcγR) and FcγRIIa (a low-affinity FcγR) [37] and it is possible that IgG2 antibodies, which do not bind to FcγRI, interfere with the binding of IgG1 antibodies to that receptor on monocytes. However, pDCs primarily express FcγRIIa [37] and IgG2 antibodies might therefore have a stimulatory rather than inhibitory role for

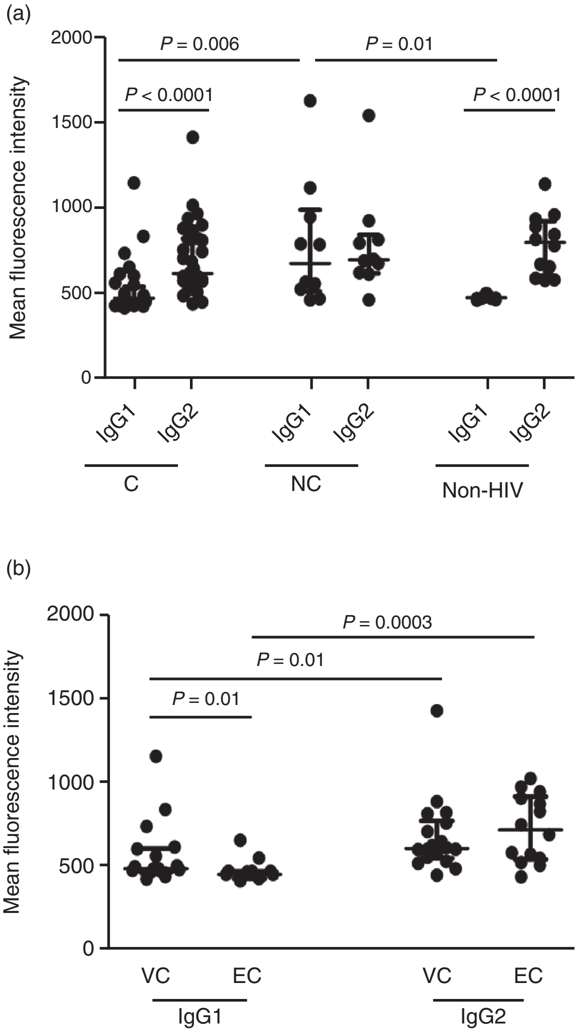

Fig. 4. FcγRIIa-binding IgG1+ and IgG2+ immune complexes in plasma from HIV controllers (C), noncontrollers (NC) and non-HIV individuals (non-HIV) (a). As values for IgG1 in HIV controllers were higher than in non-HIV individuals, virological controllers (VC) (HIV RNA < 2000 copies/ml) were compared with elite controllers (EC) (HIV RNA <50 copies/ml) (b). Plasma levels of immune complexes are reported as the mean fluorescence intensity (MFI) of IgG1 or IgG2 binding to the IIA1.6 cells.

pDCs. Indeed, activation of pDCs by immune complexes binding to FcγRIIa has been demonstrated for several antigens. For example, studies in patients with systemic lupus erythematosus have shown that pDC activation and production of interferon-alpha (IFN-α) is induced by complexes of DNA and anti-DNA IgG binding to FcγRIIa, leading to transportation of the immune complexes to endosomes where CpG DNA binds to TLR9 [36,53]. This results in the production of proinflammatory and Th1 cytokines as well as IFN-α. A similar mechanism of phagocytosis via FcγRIIa and intracellular transportation to endosomal TLR7 has also been described for immune complexes of Coxsackievirus RNA and antibody [54].

Antibodies to gp41 or gp120 are major components of plasma immune complexes in HIV patients, but these immune complexes are not associated with control of HIV infection [55]. Plasma immune complexes in HIV patients also contain HIV p24 and probably HIV RNA [56,57]. We therefore speculate that IgG antibodies to HIV Gag proteins induce the production of immune complexes containing HIV RNA and that isotype switching to IgG2 antibodies facilitates binding of these complexes to FcγRIIa on cells of the innate immune system, such as pDCs. This would permit HIV RNA to be sensed by TLR7 in pDCs, as has been described for coxsackieviruses [54]. Furthermore, antibodies to the HIV core, as opposed to envelope, would not increase antibody-enhanced infection of cells, such as macrophages [58]. Our analysis of plasma FcγRIIa-binding immune complexes did not demonstrate higher levels of IgG2+ complexes in controllers but did demonstrate more abundant IgG1 in FcγRIIa-binding immune complexes of noncontrollers than non-HIV individuals and HIV controllers despite lower IgG1 antibody responses to HIV proteins. The significance of this finding is uncertain, but IgG1 in FcγRIIa-binding immune complexes might adversely affect their interaction with antigen presenting cells, as shown for IgG3 in IgG–IgM immune complexes [39].

Vaccination with HIV p24, or components of it, has been associated with an anti-HIV effect in a variety of circumstances, but immune correlates of that effect are unclear. In addition to our previous findings [28], there have been two studies of note. Vaccination with a combination of four p24-like peptides (Vacc-4x), using strategies to increase antigen processing by cutaneous Langerhan's cells, induced weak antibody responses and long-lasting, delayed-type hypersensitivity and lymphoproliferative responses to the p24-like peptides but did not arrest disease progression after a median time of 7.3 years [59]. Furthermore, vaccination of cats with HIV p24 in Ribi/cytokine adjuvant induced cross-reactive antibodies to feline immunodeficiency virus (FIV) p24 and was associated with 78% protection from experimental infection with FIV [60], though the immunological correlate of protection from infection in the data presented was unclear. We suggest that future studies of vaccines containing HIV p24 should include an analysis of IgG antibody isotype.

We acknowledge that our study has limitations, including multiple comparisons on a small number of patients and that we have demonstrated associations rather than causation. Nevertheless, our findings are sufficiently robust to support further studies of the role of isotype switching of IgG antibodies to HIV Gag proteins in the control of HIV infection.

In summary, we have shown that isotype-switched (IgG2+) antibodies to HIV Gag proteins are associated with natural control of HIV infection, particularly in patients who do not carry HLA-B*57. We suggest that these antibodies might facilitate the activation of innate immune cells, such as pDCs, via FcγRIIa and, thereby, enhance innate immune responses against HIV and/or adaptive immune responses against Gag antigens.

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M.F., R.C., A.K. and S.K. devised the study, wrote the protocol and wrote the manuscript.

R.C., K.W., I.F., S.F., A.S., I.S. and M.K. performed the laboratory assays.

All authors viewed and approved the final version of the manuscript.

Conflicts of interest

There are no conflicts of interest.

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