Antibody-mediated control of HIV-1 infection through an alternative pathway

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Introduction

Strategies for controlling HIV-1 infection without antiretroviral therapy (ART) or depleting cellular reservoirs of HIV-1 infection, are likely to include augmentation of protective immune responses through passive or active immunization. Recently, greater attention has been paid to the role that antibodies might contribute to this process\(^1\), including augmentation of antigen presentation to T cells\(^3,4\) and synergy with innate immune responses activated via Toll-like receptor 7 (TLR7)\(^5\). Most attention has been paid to the role of IgG antibodies to HIV-1 envelope glycoproteins and their activity in virus neutralization or activation of natural killer (NK) cells to mediate antibody-dependent cell-mediated cytotoxicity (ADCC)\(^6,7\). While substantial progress has been made in developing immunotherapy for HIV-1 infection through passive immunization with combinations of monoclonal IgG antibodies to HIV-1 gp120 that exert broad neutralization activity\(^8\), such therapies might not be suitable for long-term use or deplete HIV-1 reservoirs. Development of therapeutic HIV vaccines that will enhance HIV-1-specific antibody responses alone or concurrently with HIV-1-specific T cell responses, and particularly those that target HIV-1 reservoirs, therefore remains a research priority.

The efficacy of therapeutic vaccines in enhancing antibody responses against HIV-1 envelope glycoproteins is likely to be compromised during, and after cessation of, ART, as indicated by observations that the frequency of circulating HIV-1 gp140-specific memory B cells (MBCs) and production of HIV-1 gp140 IgG antibodies in co-cultures of circulating T follicular helper (cT\(_{FH}\)) cells with MBCs is lower in patients with HIV-1 infection controlled by ART than in individuals who control HIV-1 infection without ART (controllers)\(^9,10\), and that HIV-1 gp140-specific MBCs are more abundant than normal in activated and exhausted subpopulations of MBCs in patients not receiving ART\(^11\). Furthermore, HIV patients
receiving ART also experience persistent B cell dysfunction\textsuperscript{[12]} and abnormal IgG glycosylation\textsuperscript{[13]}.

While current research is focussed on increasing the efficacy of IgG antibodies to HIV-1 envelope glycoproteins in passive and active immunization strategies, evidence is mounting that IgG antibodies to HIV-1 matrix (p17) and/or capsid (p24) proteins, encoded by \textit{Gag}, might provide an alternative pathway of antibody-mediated control of HIV-1 infection. Here, the evidence for this is presented and compared with the evidence that HIV-1 envelope glycoprotein antibodies control HIV-1 infection.

\textbf{IgG antibodies to HIV-1 capsid and matrix proteins are associated with control of HIV-1 infection}

In primary HIV-1 infection, serum HIV-1 gp41 IgG antibodies are detectable first but appear to be more effective in reducing the transmission of HIV-1 rather than in controlling HIV-1 replication\textsuperscript{[14]}. HIV-1 p24 IgG antibodies are detectable on average 5 days after HIV-1 gp41 IgG antibodies and on average 10 days before HIV-1 gp120 IgG antibodies\textsuperscript{[15]}. Numerous studies in the ‘pre-HAART era’ demonstrated that higher serum levels and avidity of HIV-1 p24 or p17 IgG antibodies were associated with slower HIV disease progression in both adults and children whereas this was not observed for HIV-1 gp120 IgG antibodies (reviewed in French et al\textsuperscript{[16]}). Furthermore, comparison of HIV patients defined as long-term non-progressors (LTNPs) with those who experienced disease progression demonstrated that absence of HIV-1 p24 or p17 IgG antibodies was associated with disease progression whereas retention of HIV-1 gp41 or gp120 IgG antibodies had little, if any, effect on disease progression\textsuperscript{[17]}

Similar observations were made in HIV controllers in comparison with non-controllers. Banerjee et al. reported that serum levels of HIV-1 p24 IgG1 antibodies were higher in elite
controllers (ECs) than non-controllers\cite{18} and both French et al\cite{19} and Tjiam et al\cite{20} reported that serum levels of HIV-1 p17 and/or p24 IgG1 and/or IgG2 antibodies were higher in HIV controllers than non-controllers and, importantly, that this was more pronounced in viraemic controllers than ECs\cite{20}. Furthermore, when the strong association of HLA-B*5701 carriage with control of HIV-1 infection was excluded, the association of HIV-1 p24 IgG1 and IgG2 antibodies with control of HIV-1 infection was only seen in viraemic controllers\cite{21}.

In contrast, serum levels of HIV-1 gp120 IgG antibodies in controllers were reported not to differ from, or to be lower than, non-controllers\cite{22,23}. However, Madhavi et al. reported that serum levels of HIV-1 gp140 IgG antibodies were higher in ECs than non-controllers\cite{24}, which had also been reported by Tjiam et al but for IgG2 and not IgG1 antibodies\cite{20}. One explanation for these findings is that HIV-1 gp140 contains the ectodomain region of gp41\cite{25} and HIV-1 gp41 IgG2 antibodies were reported to be higher in LTNPs than progressors\cite{26} and in ECs carrying ‘protective’ HLA-B alleles compared with ECs not carrying those alleles\cite{27}.

The association of HIV-1 IgG antibodies with control of HIV-1 infection has also been studied by examining antibody functional activities. Comparison of the neutralization activity of HIV-1 antibodies in HIV controllers and non-controllers in multiple studies has not demonstrated a relationship between neutralization activity and control of HIV-1 infection\cite{22,28-30}. Indeed, controllers generally exhibit low or undetectable antibodies with neutralization activity, probably reflecting low antigenic stimulation of B cells. However, LTNPs in the French ALT cohort (which contains individuals with CD4+ T counts >600/µL for at least 5 years and various HIV viral loads) who possessed IgG antibodies to the 3S motif of HIV-1 gp41 exerting neutralizing activity exhibited lower plasma HIV RNA and cellular HIV-1 DNA levels as well as higher CD4+ T cell counts\cite{31}. 
Studies of HIV-1 envelope IgG antibodies that mediate ADCC and/or NK cell activation have been inconclusive. Lambotte et al reported that HIV-1 gp120-specific ADCC responses were higher in controllers than non-controllers\[^{22}\], especially controllers not carrying HLA-B57\[^{32}\]. However, Ackerman et al. did not demonstrate a difference between ECs and non-controllers, nor for any other functional activity of HIV-1 gp120 IgG antibodies\[^{23}\]. Madhavi et al reported that HIV-1 gp140 IgG antibodies that activate NK cells and mediate ADCC were higher in ECs than non-controllers\[^{24}\] but studies from the same group demonstrated that HIV-1 gp140 IgG antibodies that activate and degranulate NK cells correlated only weakly, or not at all, with rate of CD4\(^+\) T cell decline\[^{33, 34}\].

As p24 is the major component of the HIV-1 capsid, HIV-1 p24 IgG antibodies cannot elicit virus neutralisation. HIV-1 p24 IgG antibodies also appear not to activate NK cells\[^{35}\] and, therefore, ADCC is an unlikely mechanism by which they might control HIV-1 infection. Given that IgG antibodies complexed with HIV-1 virions can be phagocytosed by plasmacytoid dendritic cells (pDCs) via Fc\(\gamma\)RIIa and augment innate responses that result in interferon-alpha (IFN-\(\alpha\)) production\[^{36}\], and that IgG antibodies complexed with capsids of some non-enveloped RNA viruses can activate pDCs via Fc\(\gamma\)RIIa to produce IFN-\(\alpha\) in humans and animals\[^{37-41}\], the most likely functional effect of HIV-1 p24 IgG antibodies is opsonisation of HIV-1 capsids (containing HIV-1 RNA), released from abortively-infected CD4\(^+\) T cells undergoing pyroptosis\[^{42}\], leading to their phagocytosis by pDCs via Fc\(\gamma\)RIIa and augmentation of IFN-\(\alpha\) production, via TLR7, that suppresses HIV-1 replication in other CD4\(^+\) T cells (Figure). This proposal is supported by observations that over 80% of plasma HIV-1 p24 is complexed with IgG antibodies in chronic HIV-1 infection\[^{43}\] and that a large proportion of HIV-1 p24 appears to be non-virion associated\[^{44}\]. In addition, infectious HIV-1 virions are isolated from less than one third of immune complex samples\[^{45}\].

To investigate the possibility that opsonophagocytic antibody responses against HIV-1 capsids might control HIV-1 infection by activating pDCs, Tjiam et al examined IgG
antibodies that opsonise particles coated with HIV-1 p24 and are phagocytosed by a pDC cell line (Gen2.2 cells) via FcγRIIa and demonstrated that this type of antibody response is higher in controllers than non-controllers, especially viraemic controllers and individuals not carrying HLA-B*5701[^20,^21]. Furthermore, such antibody responses were also associated with control of early HIV-1 infection[^46]. The effect of this type of antibody response on pDC function downstream of phagocytosis is yet to be defined.

**Antibodies to capsid and matrix proteins may elicit immune responses against HIV-1**

The association of HIV-1 p24 and p17 IgG antibodies with control of HIV-1 infection has been attributed to their being markers of CD4+ T cell ‘help’ for HIV-1 Gag-specific CD8+ T cell responses[^17]. However, several studies have provided evidence that they might act independently of T cell responses. Firstly, serum HIV-1 p17 and/or p24 IgG1 and/or IgG2 antibody levels were similar in non-controllers and controllers carrying HLA-B*5701, which is strongly associated with CD8+ T cell-mediated control of HIV-1 infection, while being higher in controllers not carrying ‘protective’ HLA-B alleles than in non-controllers[^16,^21]. Secondly, Chung et al demonstrated that plasma HIV RNA levels in South African patients with untreated HIV-1 subtype C infection inversely correlated with HIV-1 p24 IgG1 antibody levels independently of Gag-specific CD8+ T cell responses and ‘protective’ HLA-B alleles[^47]. Thirdly, a study of over 4,000 Swiss patients established to examine predictors of HIV-1 antibody neutralization breadth[^48], demonstrated that the strongest inverse correlations with plasma HIV RNA levels were serum levels of HIV-1 p17 and p24 IgG1 antibodies and, to a lesser extent, HIV-1 p24 IgG2 antibodies. These correlations were independent of CD4+ T cell counts. Interestingly, there was an inverse correlation between plasma HIV RNA levels and levels of IgG3 antibodies to HIV-1 gp41 but not HIV-1 p24.
These findings suggest that HIV-1 p17 and/or p24 IgG antibodies might contribute directly to the control of HIV-1 infection. In support of this, Tjiam et al reported that plasma HIV-1 p24-specific IgG antibodies that opsonised HIV-1 p24-coated particles and were phagocytosed by a pDC cell line (Gen2.2 cells) inversely correlated with plasma HIV RNA levels in chronic HIV-1 infection\[^{[20]}\]. In addition, the findings of a small study provided preliminary evidence that vaccine-induced HIV-1 p24 IgG antibody responses might contribute to control of HIV-1 infection. Administration of DNA vaccines encoding a fowlpox virus (FPV) vector with HIV-1 Gag-Pol and interferon-gamma (IFN-\(\gamma\)) (n=12) or HIV-1 Gag-Pol without IFN-\(\gamma\) (n=11), or a placebo (n=12), to patients with HIV-1 infection controlled by ART, demonstrated that only the vaccine including IFN-\(\gamma\) was associated with control of HIV replication after ART was ceased\[^{[49]}\]. It could not be shown that IFN-\(\gamma\) in the vaccine augmented HIV-1 Gag-specific T cell responses\[^{[50]}\] but amongst patients who received the FPV/Gag-Pol/IFN-\(\gamma\) vaccine, the most robust control of HIV-1 replication occurred in those who produced HIV-1 p24 IgG2 antibodies and carried a high affinity genotype of Fc\(\gamma\)RIIa\[^{[51]}\]. While the findings of this study must be interpreted with caution because of small patient numbers, they could illuminate novel mechanisms of antibody-mediated control of HIV-1 infection. Thus, IFN-\(\gamma\) augments IgG2 production by B cells\[^{[52]}\] and in concert with B cell receptor co-stimulation, induces IgG2 class-switching in IgM\(^+\) MBCs, which express high levels of IFN-\(\gamma\)R1\[^{[53]}\]. Furthermore, IgM\(^+\) MBCs play a role in producing non-neutralising IgG antibodies to structural proteins of some RNA viruses that control their replication, as exemplified by rotavirus\[^{[54]}\]. Stimulation of IgM\(^+\) MBCs by the FPV/HIV-1 Gag-Pol/IFN-\(\gamma\) vaccine resulting in an IgG antibody response against HIV-1 p24, including IgG2 antibodies, may therefore explain the findings of Emery et al\[^{[49, 50]}\].
Furthermore, as IgM+ MBCs enter germinal centre (GC) reactions to a greater extent than IgG+ MBCs\textsuperscript{[53, 55]}, the findings of several studies suggesting that T\textsubscript{FH} cells regulate HIV-1 p24 and p17 IgG antibody responses are of particular interest. Thus, amongst lymph node T\textsubscript{FH} cells from patients with untreated HIV-1 infection, the frequency of Gag-specific T\textsubscript{FH} cells was at least twice as high as the frequency of HIV-1 gp120-specific T\textsubscript{FH} cells\textsuperscript{[56]}, and in ECs, the frequency of HIV-1 Gag-specific cT\textsubscript{FH} cells was over four times higher than in patients with HIV-1 infection controlled by ART\textsuperscript{[10]}. Also, in South African patients with acute HIV-1 infection, the frequency of cT\textsubscript{FH} cells with a Th1 phenotype correlated with serum levels of HIV-1 p24 and p17 IgG antibodies at one year, and to a greater degree than HIV-1 gp41 or gp120 IgG antibodies\textsuperscript{[57]}. Moreover, HIV-1 p24 IgG antibodies at one year inversely correlated with HIV-1 viral load set point. An inverse correlation was also observed between HIV-1 viral load set point and HIV-1 gp41 but not HIV-1 gp120 IgG antibodies.

**Could HIV-1 p24 IgG2 antibodies complement HIV-1 p24 IgG1 antibodies in controlling HIV-1 infection?**

While IgG1 is the predominant subclass of HIV-1 IgG antibodies in chronic HIV-1 infection\textsuperscript{[23, 48]}, other IgG subclasses likely complement IgG1 antibodies in particular functional activities, as exemplified by IgG3 antibodies in the neutralisation and/or NK cell activating activity of HIV-1 gp120 IgG antibodies\textsuperscript{[23, 58]}. The findings of several studies suggest that HIV-1 p24 IgG2 antibodies might complement HIV-1 p24 IgG1 antibodies in controlling HIV-1 infection\textsuperscript{[19-21, 48, 51]}. However, this was not demonstrated in a small group (n=16) of predominantly ECs\textsuperscript{[18]}, patients with early HIV-1 infection\textsuperscript{[46]} or South African patients with chronic HIV-1 subtype C infection\textsuperscript{[47]}. Notably, the association of HIV-1 p24 IgG2 antibodies with control of HIV-1 infection was observed after vaccination\textsuperscript{[51]} or in
chronic HIV-1 infection with active viral replication, including viraemic controllers and particularly after excluding the effect of HLA-B*5701[19-21, 48]. These findings suggest that antigen stimulation of B cells is required to observe the association of HIV-1 p24 IgG2 antibodies with control of chronic HIV-1 infection, though South African patients may be an exception[47].

Investigations of how IgG2 antibodies might enhance HIV-1 p24 IgG antibody responses should focus on functional activities that are characteristic of IgG2. While IgG2 antibodies bind poorly to antigens with a low epitope density[59] and possess Fc domains that exhibit FcγR binding that is predominately limited to high affinity genotypes of FcγRIIa[60], IgG2 aggregates and forms covalent dimers more than IgG1, through greater inter-chain disulphide bond connectivity[61, 62], and is normally the predominant IgG subclass in plasma IgM-IgG immune complexes, where it enhances binding to mononuclear leucocytes[63]. Therefore, HIV-1 p24 IgG2 antibodies might enhance HIV-1 capsid/antibody complex formation and their phagocytosis via FcγRIIa. Investigations of this demonstrated that FcγRIIa-binding immune complexes isolated from plasma of HIV controllers exhibited a much higher proportion of IgG2 than IgG1, which was similar to HIV seronegative subjects, whereas plasma FcγRIIa-binding immune complexes from non-controllers did not[19]. Of note, patients with chronic HIV-1 infection exhibit much lower serum IgG2/IgG1 ratios than normal[12]. However, using HIV-1 p24-coated beads opsonised ex vivo with HIV-1 p24 IgG antibodies from patients with HIV-1 infection, Tjiam et al could not demonstrate that IgG2 antibodies complemented IgG1 antibodies in phagocytosis by pDCs via FcγRIIa[20].

Conclusions and future perspectives

The evidence that HIV-1 p17 and/or p24 IgG antibodies are associated with natural
control of HIV-1 infection is at least as convincing as the evidence that HIV-1 gp120 and/or HIV-1 gp41 IgG antibodies control HIV-1 infection. However, that evidence is largely correlative in nature and studies to examine the effect of augmenting HIV-1 p24 IgG antibody responses, by passive or active immunization, on the replication of HIV-1, or simian-human immunodeficiency virus infection in non-human primates, are required. Active immunization using vaccination strategies and/or vaccines that induce strong HIV-1 p24 IgG antibody responses\textsuperscript{[64, 65]} is a potential means of doing this.

Although HIV-1 p17 and/or p24 IgG antibodies are unlikely to mediate neutralization of HIV-1 or ADCC of HIV-1 infected cells, it is feasible that they mediate an opsonophagocytic antibody response against virion- and cell-free HIV-1 capsids (containing HIV-1 RNA) that augments pDC and IFN-\(\alpha\) responses against HIV-1-infected cells. Further investigation of (a) the proposed role of IgM\(^{+}\) MBCs in generating an effective HIV-1 p24 IgG antibody response, (b) the effects of HIV-1 p24 IgG antibody responses on pDC function downstream of phagocytosis, and (c) the proposed activities of HIV-1 p24 IgG2 antibodies in enhancing HIV-1 capsid/antibody complex formation and binding to Fc\(\gamma\)RIIa, may strengthen the hypothesis that there is an alternative pathway of antibody-mediated control of HIV-1 infection and lead to novel therapeutic approaches for controlling HIV-1 infection, including vaccination strategies that enhance antibody and T cell responses against Gag-encoded antigens concurrently.

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Diagramatic representation of a proposed alternative pathway for antibody-mediated control of HIV-1 infection. HIV-1 capsids released from virions into the cytoplasm of CD4+ T cells enter the extracellular environment after abortive infection and pyroptosis of the cell and are bound by HIV-1 p17 and/or p24 IgG antibodies. HIV-1 capsid/antibody complexes are phagocytosed by pDCs via FcγRIIa and HIV-1 RNA released from capsids binds to TLR7 in the endosomal compartment of pDCs. The downstream effects of HIV-1 RNA binding to TLR7 leads to the production of type I interferons (T1 IFN), particularly IFN-α. IFN-α produced by pDCs induces interferon-stimulated genes (ISG) in CD4+ T cells productively infected with HIV-1 leading to suppression of HIV-1 replication (indicated by the green lines).
References


