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NOTES

Immunoglobulin and Complement Complexes in Blood following Infection with Human Immunodeficiency Virus Type 1

XUAN-XIAN PENG,[1] MARK A. WAINBERG,[2,3] YUZHEN TAO,[2] AND BLUMA G. BRENNER[2,4]*

McGill AIDS Centre, Lady Davis Institute-Jewish General Hospital,[2] and Departments of Experimental Surgery[4] and Medicine,[3] McGill University, Montreal, Quebec, Canada, and Department of Biological Science and Technology, Nanchang University, Nanchang, People's Republic of China[1]

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Freely soluble and complexed plasma immunoglobulin A (IgA), IgG, IgM, C1q, C3, and factor B in 36 human immunodeficiency virus type 1 (HIV-1)-seronegative controls, 69 asymptomatic HIV$^+$ subjects, and 117 individuals with symptomatic HIV-associated disease were characterized. Levels of free and complexed IgG and IgA, and to a lesser extent free C1q and complexed IgM, increased with HIV-1 infection. In stark contrast, both HIV$^+$ groups showed three- to sixfold declines in complexed C3, C1q, and factor B levels. The asymptomatic HIV$^+$ population showed declines in levels of C3-bound IgA, IgG2, and IgG4 complexes. The symptomatic group showed reductions in C3-complexed IgM, IgA, IgG2, and IgG4 levels. HIV infection is associated with complement-deficient immune complexes.

How human immunodeficiency virus type 1 (HIV-1) evades the humoral immune system remains a major outstanding question in AIDS research. Paradoxically, B-cell activation and impaired B-cell responses are coexistent features of AIDS (3, 9, 12). Cytokines, T-cell subsets, and non-T accessory cells have been shown to play participatory roles in B-cell activation (1, 3, 11, 14). Hypergammaglobulinemia may reflect nonspecific B-cell activation, antigen-driven B-cell responses secondary to HIV-1 and/or microbial challenge, or heightened autoimmune reactions (3, 4, 6, 8, 9, 16). Few studies have attempted to explain the defect in B-cell functional response following HIV infection. The present cross-sectional investigation has characterized the heterogeneity in humoral immunoglobulin (Ig) and complement complexes at different stages of HIV infection.

Class diversity in antibody response has likely evolved to cope with different antigenic challenges at different anatomical sites. The immunoglobulin isotypes of HIV-directed antibodies and their specificities to structural and regulatory viral proteins have been characterized in some detail (2–4). However, little is known about how effectively these antibodies form complexes, bind complement, and clear antigen in infected individuals. Ig classes and subclasses vary considerably not only in their antigenic specificities and avidities but also in their innate capacities to bind complement and initiate classical and/or alternate complement cascades (10, 21). Failure to clear infectious immune complexes has been postulated to be a mechanism that leads to viral persistence in which viral antibodies enhance rather than neutralize infection (10, 18, 20).

We obtained plasma samples from 183 different HIV-1-seropositive individuals and 36 seronegative healthy controls with informed written consent. Sixty-nine HIV$^+$ subjects were classified as clinically asymptomatic (Centers for Disease Control and Prevention group II [CDC-2]) with no disease manifestations beyond HIV seropositivity. The remaining 117 individuals had AIDS-defining opportunistic infections (CDC-4). Plasma samples were subjected to 2% polyethylene glycol (PEG) fractionation (PEG 8000; J. T. Baker Co., Phillipsburg, N.J.). Radial immunodiffusion analyses determined the concentrations of freely soluble and complexed IgA, IgG, IgM, C3, C1q, and factor B. C3-binding enzyme-linked immunosorbent assays (ELISAs) were developed for the detection of Ig class-specific, complement-bound immune complexes in unfractionated plasma (22). Polystyrene 96-well plates (Maxisorp Immunoplates; Nunc, Roskilde, Denmark), coated with 100 μl of 10-μg/ml anti-human C3, were incubated first with plasma samples; then with horseradish peroxidase-conjugated $F(ab)_2$ antibody fragment-specific α, γ, γ1, γ2, γ3, γ4, or μ immunoglobulin chains (Organon Teknika Corp., West Chester, Pa.); and finally with *o*-phenylenediamine, a peroxidase substrate.

Analysis of variance and post hoc Tukey tests were used to examine for differences in various humoral variables among HIV$^-$ and HIV$^+$ subgroups. Because of wide variations in IgA and IgG levels, a logarithmic transformation was applied to all data before statistical analysis (Systat, Evanston, Ill.). For ease of interpretation, all data have been expressed as antilog values.

Concentrations of Ig classes in PEG-soluble and PEG-insoluble plasma subfractions for seronegative control (CDC-0), HIV$^+$ asymptomatic (CDC-2), and AIDS (CDC-4) groups are summarized in Table 1. Levels of freely soluble and complexed IgA and IgG rose in both asymptomatic and symptomatic HIV$^+$ groups (Table 1). To a lesser extent, levels of complexed IgM increased in the asymptomatic HIV$^+$ group only. Interestingly, HIV infection favored an environment of either IgG or IgA hypergammaglobulinemia. Whereas IgG represented the primary antibody response in seronegative persons (60 to 84% of the circulating Ig), it composed 15 to 94% and 7 to 95% of the circulating and complexed Ig in the CDC-2 and CDC-4 groups, respectively. IgA composed 3 to 67% of the circulating Ig and 0 to 84% of the complexed Ig in these two HIV$^+$ groups. There were strong negative associations between the

* Corresponding author. Mailing address: Lady Davis Institute-Jewish General Hospital, 3755 Cote Ste Catherine Rd., Montreal, Quebec, Canada H3T 1E2. Phone: (514) 340-8260. Fax: (514) 340-7537.

TABLE 1. Concentrations of Ig and complement factors in PEG-soluble or PEG-insoluble plasma among HIV$^-$ and HIV$^+$ groups[a]

Ig or complement factor in plasma subfraction	Avg concn (range) in the following group[b]:			F value[c]
	HIV$^-$, CDC-0	HIV$^+$, CDC-2[d]	HIV$^+$, CDC-4[e]	
Free				
IgA	224 (113–415)	249 (62–1,468)	352 (80–1,890)[f,g]	12[h]
IgG	1,094 (667–1,556)	1,557 (354–4,481)[f]	1,587 (80–5,536)[f]	9[h]
IgM	110 (97–144)	118 (88–222)	114 (88–265)	2
C3	112 (77–204)	121 (73–225)	125 (52–277)	2
C1q	7 (4–10)	13 (7–44)[f]	16 (4–44)[f]	10[h]
Complexed				
IgA	3 (1–6)	6 (1–359)[f]	7 (0–263)[f]	14[h]
IgG	13 (4–36)	41 (8–273)[f]	35 (4–525)[f]	21[h]
IgM	9 (7–11)	11 (4–43)[f]	9 (4–39)[g]	7[h]
C3	11 (2–104)	2 (1–10)[f]	2 (1–56)[f]	124[h]
C1q	15 (4–33)	4 (3–10)[f]	5 (2–13)[f]	69[h]
FB[i]	7 (3–13)	2 (1–10)[f]	3 (1–34)[f]	21[h]

[a] PEG-soluble plasma contained free Ig and complement factors; PEG-insoluble plasma contained complexed Ig and complement factors.
[b] Data are in milligrams per deciliter.
[c] From one-way analysis of variance.
[d] CD4 cell number, 390 ± 35 cells per μl.
[e] CD4 cell number, 150 ± 19 cells per μl.
[f] Significantly different from value for CDC-0 seronegative group by Tukey test.
[g] Significantly different from value for CDC-2 asymptomatic group by Tukey test.
[h] $P < 0.001$.
[i] FB, factor B.

levels of IgA and IgG in HIV-1$^+$ groups ($r = -0.97$ and -0.69 for uncomplexed and complexed constituents, respectively).

The circulating concentrations of complement components involved in both classical (C1q and C3) and alternate (C3 and factor B) complement pathways are also summarized in Table 1. The amounts of freely soluble C3 were invariable among the seronegative, CDC-2, and CDC-4 groups. Levels of soluble C1q in plasma marginally increased with HIV infection. In stark contrast, the asymptomatic and the symptomatic HIV$^+$ populations showed similar three- to sixfold reductions in concentrations of C3, C1q, and factor B within complexes compared with corresponding levels of complexed complement in the seronegative control group (Table 1).

Since the HIV$^+$ groups showed wide variations in their CD4$^+$ cell numbers, individuals were stratified into three groups on the basis of blood CD4$^+$ cell counts. Subjects with early disease having CD4 counts above 500 cells per μl had free and complexed IgA levels that were within normal limits and significantly lower than those for the other two groups (Table 2). Nevertheless, all three HIV$^+$ groups showed marked declines in levels of complexed C1q, C3, and factor B relative to the seronegative control group. Indeed, the level of complexed C1q in the group having >500 cells per μl was lower than those in the other two groups with more advanced disease. This suggests that complement-deficient complexes arise before the onset of opportunistic infection. It appears that changes in complexed complement factors arise early in infection and are likely to be a direct consequence of HIV infection per se.

Ig subclasses differ markedly in their capacities to bind and activate complement (10, 21). Antigen-free IgG or IgM does not bind C1q. While antigen-bound pentameric IgM binds C1q efficiently, a single Fcγ region in IgG is insufficient to bind C1q. C1q can bind to aggregated IgG within immune complexes. However, IgG subclasses differ in their abilities to initiate the classical cascade. IgG3 efficiently activates complement, IgG1 activates it somewhat less efficiently, IgG2 activates it poorly, and IgG4 does not activate it at all. Antigen-bound IgA and IgG4 complexes activate the far less efficient alternate complement pathway. Since C3 is a component common to both complement pathways, we used anti-C3-conjugate ELISAs with unfractionated plasma to monitor overall levels of class-

TABLE 2. Average levels of free and complexed Ig and complement in HIV$^+$ individuals stratified on the basis of their CD4$^+$ cell numbers in blood

Ig or complement factor in plasma subfraction	Avg concn in patients with the following no. of CD4$^+$ cells per μl of blood:[a]			F value[b]
	>500[c]	200–500[d]	<200[e]	
Free				
IgA	205	294	357[f,g]	9[h]
IgG	1,625	1,645	1,582	0
IgM	116	113	116	0
C3	121	124	126	0
C1q	13	11	13	1
Complexed				
IgA	4	7[f]	7[f]	3[i]
IgG	45	45	34	2
IgM	10	10	9	1
C3	2	2	2	1
C1q	3	4[f]	5[f]	7[j]
FB[k]	2	2	2	0

[a] Data are in milligrams per deciliter.
[b] From analysis of variance. The F value for the CD4 cell numbers of the groups was 311 ($P < 0.001$).
[c] Population size, 29 patients; CD4 cell number, 632.
[d] Population size, 46 patients; CD4 cell number, 329.
[e] Population size, 103 patients; CD4 cell number, 63.
[f] Significantly different from value for group with >500 cells per μl.
[g] Significantly different from value for group with 200 to 500 cells per μl.
[h] $P < 0.001$.
[i] $P < 0.05$.
[j] $P < 0.01$.
[k] FB, factor B.

TABLE 3. Average levels of C3-conjugated Ig-class-specific complexes in plasma of HIV$^-$ and HIV$^+$ groups[a]

C3-bound Ig complex	Avg ELISA absorbance value (± SEM) for the following group:			F value[b]
	HIV$^-$ CDC-0	HIV$^+$ CDC-2	HIV$^+$ CDC-4	
C3-binding IgA	0.59 ± 0.05	0.29 ± 0.04[c]	0.41 ± 0.03[c,d]	12[e]
C3-binding IgG	0.66 ± 0.07	0.47 ± 0.05[c]	0.49 ± 0.03	4[f]
C3-binding IgG1	0.32 ± 0.03	0.37 ± 0.03	0.40 ± 0.04	0
C3-binding IgG2	0.87 ± 0.11	0.51 ± 0.06[c]	0.49 ± 0.04[c]	19[e]
C3-binding IgG3	0.30 ± 0.03	0.39 ± 0.05	0.40 ± 0.04	1
C3-binding IgG4	2.08 ± 0.23	0.93 ± 0.10[c]	0.86 ± 0.06[c]	22[e]
C3-binding IgM	0.39 ± 0.03	0.41 ± 0.03	0.30 ± 0.01[c,d]	8[e]

[a] Anti-C3 conjugate Ig-class-specific ELISAs were performed on unfractionated plasma samples.
[b] From one-way analysis of variance.
[c] Significantly different from value for CDC-0 group by Tukey test.
[d] Significantly different from value for CDC-2 group by Tukey test.
[e] $P < 0.001$.
[f] $P < 0.05$.

specific, antigen-bound immune complexes in seronegative and seropositive groups (Table 3).

Both asymptomatic and symptomatic HIV$^+$ groups demonstrated reduced levels of C3-conjugated IgA and IgG complexes, despite increases in circulating and complexed IgA and IgG levels (Table 3). The level of C3-complexed IgA in the CDC-4 symptomatic group was significantly greater than that in the asymptomatic group. The reductions in C3-complexed IgG levels were limited to the IgG2 and IgG4 subclasses. There were no differences in C3-bound IgG1, IgG2, IgG3, and IgG4 levels between the CDC-2 and CDC-4 groups. In contrast, the relative amount of C3-conjugated IgM complexes was sustained in the HIV$^+$ asymptomatic group but declined in the symptomatic group (Table 3). Thus, the presence of an AIDS-defining event in patients was associated with increased levels of C3-bound IgA and decreased levels of C3-bound IgM.

Few studies have investigated the role of complement in HIV infection. We have shown that individuals with HIV infection show normal to elevated synthesis of complement factors. The authors of other published studies have concluded that complement-mediated processes are normal to elevated in HIV-infected individuals on the basis of circulating concentrations of serum complement components and their activated fragments (17, 20). A major finding of this study indicates that immune complexes in infected individuals were of a complement-poor subtype. There were profound reductions in the relative amounts of these complement factors associated with immune complexes and in particular antigen-bound complexes. Our demonstration of the presence of complement-deficient complexes in a large HIV$^+$ population confirms observations made in two earlier studies (5, 15) but disagrees with observations by another group (13).

We can only speculate as to the source of complement-deficient complexes. They may arise secondarily to Ig isotypic imbalance. For example, IgA complexes, which activate the less efficient alternate complement pathway, can compete with IgG complexes for complement factors. In this regard, others have shown that IgA hypergammaglobulinemia is a surrogate marker and negative prognostic indicator that combines with CD4 cell number in charting clinical progression in infected individuals (4). There is also evidence that complexes in AIDS patients bear C3b1 fragments and not C1q, activating the alternate but not the classical pathway (20). This may explain the reversed distribution of C1q in free and complexed states in seronegative versus, seropositive donors. A conflicting report suggests a direct relationship between disease progression and activation of the classical complement pathway (17).

Nonprimed B cells make IgM in a T-cell-independent fashion. Ig class switching is governed by cytokine signals from T-helper subsets and natural killer (NK) cells. For example, interleukin-2 and interleukin-10 have been shown to stimulate IgG1, IgG2, IgG3, and IgA synthesis, while interleukin-4 favors IgE, IgG1, and IgG4 synthesis (11). Our findings showing alterations in IgA, IgG2, IgG4, and IgM suggest complex changes in humoral response with HIV infection.

HIV-1, its envelope proteins, and virally infected cells have been shown to directly bind and/or activate complement in an antibody-independent fashion (7, 10, 19). For example, binding of immunodominant regions of gp41 to gp41 antibodies can be abrogated following treatment with serum or purified C1q. It is possible that uncomplexed complement-bound HIV can target virus to cells bearing receptors for C3 fragments, e.g., follicular dendritic cells. Alternatively, the failure of HIV-carrying immune complexes to bind complement may also have negative clinical implications. If infectious immune complexes don't bind complement, viral persistence can result through infection of cells having class-specific Fc receptors. HIV infection may also lead to antigen-specific responses that do not evoke complement-mediated responses. For example, HIV infection results in preferential expansion of IgG1 and IgG3 over IgM resulting in readily dissociable complement-bound complexes (2, 3). Thus, hypergammaglobulinemia may not necessarily elicit strong humoral responses.

Our results show that HIV infection leads to complement-deficient immune complexes. Monitoring of complexed and complement-bound complexes may provide novel information concerning changes in humoral immune response with infection. We intend to relate changes in complement-bound complexes to viral antigenemia and viral RNA burden. These findings suggest a need for novel therapeutic strategies to modulate humoral immune responses in AIDS (3, 21).

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