Anti-F(ab')$_2$ Antibodies in Thrombocytopenic Patients at Risk for Acquired Immunodeficiency Syndrome

Ji-Ren Yu,* Evelyne T. Lennette,‡ and Simon Karpatkin*
*Department of Medicine, New York University Medical School, New York, New York 10016; and ‡Virolab, Inc., Berkeley, California 94710

Abstract

22 homosexual or narcotic addict patients at risk for acquired immunodeficiency syndrome (AIDS) or with AIDS, were studied for the presence of antiimmunoglobulin antibodies and circulating immune complexes (20 were thrombocytopenic, 6 had AIDS). Circulating immune complex levels were 10-fold higher than levels in normal subjects. IgG anti-F(ab')$_2$ antibodies were noted in homosexual as well as narcotic addict patients. Of 16 homosexual patients, 7 had IgG anti-F(ab')$_2$ antibody of moderate to marked titer with broad reactivity against autologous, homologous, and control F(ab')$_2$ fragments. Three others demonstrated limited reactivity against one or two F(ab')$_2$ fragments. The remaining six patients were negative. Six of six narcotic addict patients had IgG anti-F(ab')$_2$ antibody, five with limited reactivity, one with broad reactivity. In contrast, neither elevated circulating immune complexes nor anti-F(ab')$_2$ antibodies were detectable in six autoimmune thrombocytopenic patients.

Anti-F(ab')$_2$ antibody could be affinity purified from serum or circulating immune complexes. Anti-F(ab')$_2$ reactivity correlated with circulating immune complex levels, $r = 0.83$, $P < 0.01$.

Introduction

Individuals at risk for the acquired immunodeficiency syndrome (AIDS):[1] sexually active homosexuals (1–3), narcotic addicts (4), and multiply transfused hemophiliacs (5, 6) develop a new syndrome of idiopathic thrombocytopenic purpura (ITP), which is clinically indistinguishable from classic autoimmune thrombocytopenic purpura (ATP) (7). However, immunologic studies (1–4, 6) reveal significant differences between the new syndrome(s) and classic ATP. Thrombocytopenic patients at risk for AIDS have markedly elevated platelet-bound IgG and complement (C3C4) compared with ATP patients, markedly elevated incidence and level of circulating immune complexes (determined by the polyethylene glycol [PEG] precipitation method) compared with ATP patients, and serum antibody against the AIDS-related retrovirus. Recent studies (2) evaluating the immunologic mechanisms involved have suggested that this disorder may be mediated by circulating immune complexes in homosexual ITP (HSITP); and by circulating immune complexes as well as specific antiplatelet 7S IgG in narcotic addicts (NITP) (4).

Our present report describes studies on immune complexes in patients at risk for AIDS and reveals the presence of Ig anti-F(ab')$_2$ antibodies in the serum and circulating immune complexes of these patients.

Methods

Population. The population studied for anti-F(ab')$_2$ antibody included 16 sexually active homosexuals (10 with thrombocytopenia alone, 4 with thrombocytopenia and AIDS [3 with Kaposi's sarcoma, 1 with *Pneumocystis carinii* pneumonia], 2 with AIDS [Kaposi's sarcoma] and normal platelet counts); 6 narcotic addicts with thrombocytopenia; 7 classic female ATP patients; and 5 control subjects. The clinical manifestations presented by these patients are essentially the same as those described in previous publications (1–4, 7).

Procedures. Viral studies for detection of antigen as well as antibody were performed for Epstein-Barr virus (EBV), cytomegalovirus (CMV), herpes simplex virus (HSV), rubeola virus, and adenoviruses (ADE) using standard indirect immunofluorescent techniques.

Antigen-containing platelets or PEG-precipitated immune complexes were washed twice with centrifugation at 3,000 *g* in 0.01 M phosphate-buffered saline (PBS), pH 7.4, containing 1 mg/ml bovine serum albumin and spotted on glass slides, which were divided into two groups. One group was treated with 0.1 M glycine buffer, pH 3.0 for 15 min, and rinsed twice with PBS to remove dissociated antibodies. Both treated and untreated fractions were fixed in acetone and tested for viral antigens using fluorescein isothiocyanate-conjugated rabbit antisera specific for each of the moieties to be tested.

Antibody specificities in sera or in immune complex precipitates were tested as follows: Each sample was diluted in 0.1 M glycine buffer, pH 3.0 at 1:2 or 1:8 and heated at 56°C for 30 min to dissociate endogenous complexes. The pH of each sample was adjusted to pH 7.0 and tested for antibody activity. Two standard serologic procedures were used, indirect immunofluorescence antibody staining and immune adherence hemagglutination (8).

Platelet eluates were prepared from 1×10^8 washed platelets (9) suspended in PBS. 2 ml of ether was added and the sample vigorously shaken for 2 min before being incubated in a 37°C waterbath for 30 min with shaking. The sample was then centrifuged for 15 min at 2,500 *g*, and the eluate (aqueous solution) removed with a Pasteur pipette and stored at −20°C prior to use.

Circulating immune complexes were estimated by a modified PEG precipitation procedure (10). 50 μl of test serum was added to 150 μl of

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Dr. Yu is a Visiting Scientist from the First Teaching Hospital, Beijing Medical College, Beijing, China. Address reprint requests to Dr. Karpatkin.

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1. *Abbreviations used in this paper:* ADE, adenovirus; AIDS, acquired immunodeficiency syndrome; ATP, autoimmune thrombocytopenic purpura; CMV, cytomegalovirus; EBV, Epstein-Barr virus; HSITP, homosexual ITP; HSV, herpes simplex virus; ITP, idiopathic thrombocytopenic purpura; KLH, keyhole limpet hemocyanin; NITP, narcotic addicts with ITP; PEG, polyethylene glycol; PMSF, phenylmethylsulfonyl fluoride.

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0.2 M Na_2EDTA, pH 7.4. 1 ml of 6% PEG in 0.1 M sodium borate buffer, pH 8.4, was added and incubated at 4°C overnight. The precipitate was centrifuged at 3,000 *g* at 4°C for 10 min and the supernatant discarded. The precipitate was then washed twice in 5% PEG in borate buffer, and dissolved in 200 μl PBS containing 0.02% sodium azide. The solution was assayed for protein using the Lowry procedure. When subjected to electrophoresis and stained for protein with amido black, semiquantitative estimation of protein peaks for IgG, IgM, and complement components (determined by immunoelectrophoresis) represented ~85–90% of stainable material. The presence of IgG, IgM, and C3C4 was independently confirmed with highly specific solid-phase immunoassays (4). The mean value of circulating immune complexes for nine healthy control subjects was 0.21±0.20 (2 SD) mg/ml.

Purified IgG was prepared from crude serum globulin fractions obtained from 50% saturated ammonium sulfate precipitation followed by dialysis against 0.1 M phosphate buffer, pH 7.6. The sample was then applied to a diethylaminoethyl-52 column, equilibrated with the same buffer. The flow-through material contained purified IgG.

Serum fractions enriched with IgM were obtained after gel-filtration of serum on a G-200 Sephadex column equilibrated with PBS by utilizing the void volume fraction.

$F(ab')_2$ fragments were prepared by pepsin digestion of IgG at pH 4.0 (11), followed by dialysis against PBS plus 0.01% sodium azide, followed by affinity chromatography on an insoluble staphylococcal protein A column equilibrated in the same buffer. The nonadherent material is purified $F(ab')_2$ as determined by sodium dodecylsulfate-polyacrylamide gel electrophoresis (10), as well as a solid-phase immunoassay performed with a rabbit anti-human IgG-Fc antibody.

Anti-$F(ab')_2$ IgG activity was measured by solid-phase immunoassay. 40 μl of a 5-μg/ml solution of $F(ab')_2$ fragments is applied to 12-microtiter wells (in duplicate), incubated at room temperature for 1 h, and then washed three times with PBS-azide containing 1% bovine serum albumin (1% BSA-PBS). 12 serial twofold dilutions of 40 μl of purified IgG in 1% BSA-PBS (starting with 5 μg/ml) were then applied to each well and allowed to incubate for 1 h. Nonadherent antibody was washed three times with 1% BSA-PBS, and 40 μl of radioactive staphylococcal protein A (30 mCi/mg, Amersham Corp., Arlington Heights, IL), ~150,000 cpm, applied to each well for 1 h. The wells were then washed three times as above and assayed for radioactivity. In some experiments, nonradioactive protein A was added to the $F(ab')_2$ fragments to block any possible residual Fc domains by addition of 40 μl of protein A (2 μg/ml). In each experiment: (*a*) patient IgG was run against patient $F(ab')_2$ fragment as well as control $F(ab')_2$ fragment; (*b*) Control IgG was run against autologous or homologous control $F(ab')_2$, as well as patient $F(ab')_2$. The counts per minute obtained for each serial dilution of patient IgG against patient $F(ab')_2$ were divided by the counts per minute obtained for control IgG against autologous control $F(ab')_2$, and a ratio obtained. Values > 1.3 were considered positive, since this reflects a 2-SD change of replicate measurements made on the same IgG vs. $F(ab')_2$. A positive result was defined as the highest ratio obtained by two consecutive dilutions, both of which are positive (>1.3).

Anti-$F(ab')_2$ IgM activity was measured by using 40-μl aliquots of the IgM-enriched fraction of serum, nonradioactive protein A (2 μg/ml) to block any contaminating IgG (<4% of total IgM + IgG as determined by immunoassay [4]), rabbit anti-human IgM (heavy chain specific, diluted 1:800, Cappel Laboratories, Cochranville, PA), and ^{125}I-protein A. The sensitivity of the IgM assay was 0.5 ng per well.

Affinity purification of anti-$F(ab')_2$ from serum IgG was accomplished by coupling purified $F(ab')_2$ to a solid-matrix Affi-gel column (Bio-Rad Laboratories, Richmond, CA). Affi-gel 10 was washed on a scintered glass filter with ice-cold isopropanol, followed by ice-cold distilled water, as recommended by the manufacturer. $F(ab')_2$ was dialyzed against 0.1 M 2[(*N*-morpholino)ethanesulfonic acid] buffer, pH 6.5 containing 0.1 mM phenylmethylsulfonyl fluoride (PMSF) overnight. 1 ml of $F(ab')_2$ (5 mg/ml) was then incubated with 1 ml of packed Affi-gel 10 overnight. Unattached reactive sites were neutralized with 1 M Tris-HCl buffer, pH 6.5 for 1 h and then washed. A 1-ml column was prepared from a plastic syringe, and IgG applied to the column with the effluent reapplied two times. The column was then washed extensively with 0.1 M $NaHCO_3$, pH 8.0, until the optical density reading of the effluent at 280 μm was zero. Bound antibody was then eluted with 0.1 M glycine buffer, pH 2.5, and the effluent fractions neutralized immediately with $NaHCO_3$. The peak fractions were dialyzed against water, and lyophilized until further use.

Affinity purification of $F(ab')_2$ from immune complexes was attained with the following procedure: Affi-gel coupled to $F(ab')_2$, as above, was washed with glycine buffer, pH 2.5, containing 0.1 M PMSF until the wash solution had an optical density at 280 μm of zero and pH of 2.5. The isolated immune complex precipitate was dissolved in 0.1 M glycine buffer, pH 2.5, containing protease inhibitors: 0.1 mM PMSF, 5 mM EGTA, and 10 mM benzamidine. The acidified immune complex solution and Affi-gel coupled to $F(ab')_2$ were mixed 1:1 by volume and dialyzed overnight against 0.1 M $NaHCO_3$, pH 8.0, containing protease inhibitors. The pH of the suspension in the dialysis sac should be 8.0. By this maneuver, the solid-phase $F(ab')_2$ competes for the solubilized anti-$F(ab')_2$ IgG of the immune complex as the pH is raised. The contents were then poured into a plastic syringe column and washed with 0.1 M $NaHCO_3$ plus protease inhibitors until the effluent had an optical density reading of zero. The bound anti-$F(ab')_2$ antibody was then eluted as above with 0.1 M glycine buffer, pH 2.5, and neutralized with $NaHCO_3$.

Kappa and lambda light chains of affinity-purified $F(ab')_2$ antibody were detected by solid-phase immunoassay using rabbit anti-kappa chain antibody or rabbit anti-lambda chain antibody at a dilution of 1:300 (Calbiochem-Behring Corp., American Hoechst Corp., San Diego, CA), and ^{125}I-protein A, as in the assay cited above for anti-$F(ab')_2$ reactivity.

Results

The presence of "anti-antibodies" was suggested from preliminary studies designed to analyze platelets and immune complexes for viral antigens, to which they had been exposed, and their respective antibodies. These are cited below.

Viral studies of platelets. Preliminary viral detection studies of fixed washed platelet preparations of six HSITP patients revealed absence of CMV, HSV, or ADE antigens on their platelets (under conditions wherein positive controls reacted with the test antisera), despite the presence of antiviral antibodies for these antigens in their sera: CMV antibody titer (range, 1:32–1:256); HSV (1:32–1:256); ADE (<1:8–1:64); EBV (1:320–1:640). Similarly, platelet eluates of eight HSITP patients were negative for antibodies against EBV, CMV, HSV, or ADE.

Viral studies of immune complexes. As with the platelet preparations, viral antigens for EBV, CMV, HSV, and rubeola virus were absent on four fixed immune complex preparations of HSITP patients. However, anti-viral antibody analysis of the same immune complexes did reveal antibodies against EBV, CMV, HSV, and rubeola virus, that correlated with the presence of these antibodies in their respective sera (Table I). No such correlation was noted with preparations from three healthy control subjects whose immune complex preparations were negative in the presence of positive serum titers.

These observations suggested that some immune complexes may be composed of antibodies directed against other antibodies.

Anti-$F(ab')_2$ IgG antibodies in homosexual patients. When purified IgG from 10 HSITP patients (A–V) and two Kaposi's sarcoma patients with normal platelet counts (E, F) was tested against a panel of $F(ab')_2$ fragments from four HSITP patients (A–D) and two patients with Kaposi's sarcoma alone (E, F) moderate to marked anti-$F(ab')_2$ reactivity was noted with the IgG fractions of five patients (A–I) (Table II). This was associated with fairly broad reactivity, wherein antibody was directed against autologous as well as allogeneic $F(ab')_2$, and against control as

*Table I. Antiviral Antibody Titers in Sera and Immune Complexes of Homosexual Thrombocytopenic Patients and Control Subjects**

	Patients				Controls		
Virus	P1	P2	P3	P4	C1	C2	C3
HSV							
serum	128	<8	512	128	512	32	128
complex	128	<8	<8	128	<8	<8	<8
CMV							
serum	2,048	256	256	128	32	<8	<8
complex	2,048	<8	128	128	<8	<8	<8
EBV(VCA)							
serum	1,280	5,120	10,240	640	320	320	80
complex	2,560	20,480	20,480	1,280	<20	<20	<20
EBV(EBNA)							
serum	160	160	320	80	80	80	80
complex	320	1,280	640	320	<5	<5	<5
Rubeola							
serum	64	256	64	32	ND	ND	ND
complex	256	512	128	8			

VCA, viral capsid antibody; EBNA, Epstein Barr nuclear antigen; ND, not done. * Reciprocal viral titers are given for patients (P1–P4) and controls (C1–C3) in serum samples as well as immune complexes obtained from the same serum samples. Immune complex precipitates were diluted to the same volume of serum from which they were obtained.

well as patient $F(ab')_2$. Two IgG fractions (R, S) demonstrated limited reactivity against one or two of the panel of four to six $F(ab')_2$ fragments. Three IgG fractions (T–V) demonstrated no reactivity. Similar results were obtained with the two patients with Kaposi's sarcoma (E, F). Negative results were obtained when six IgM-enriched fractions were tested against the same panel of $F(ab')_2$ fragments, using a rabbit anti-IgM antibody capable of detecting 0.5 ng of IgM anti-$F(ab')_2$ reactivity per well (data not shown). Negative results were also obtained for rheumatoid factor when the sera of 10 patients were tested by the routine clinical laboratory at our institution.

Four patients with AIDS and thrombocytopenia were also studied (Table III). Patient II demonstrated broad reactivity against the entire panel of $F(ab')_2$ fragments, which included three patients with Kaposi's sarcoma and ITP (FF, GG, HH), one narcotic addict with ITP (X) and two controls (G, H). Patient II's immune complex was dissociated at low pH and affinity purified on solid phase $F(ab')_2$ of patient GG (see Methods). The

*Table II. Binding Ratios of Homosexual IgG to $F(ab')_2$ Fragments of Homosexual Patients and Control Individuals Compared to Binding of Control IgG to Control $F(ab')_2$ Fragments**

$F(ab')_2$ fragments	Purified IgG immunoglobulin of homosexuals												Control
	A	B	C	D	I	R	S	T	U	V	E	F	G
A	6.6	2.6	1.8	4.0	1.4	0.8	1.1	0.8	0.9	0.9	1.5	1.5	1.1
B	6.6	2.5	1.8	2.8	1.2	—	—	—	—	—	1.3	1.3	0.9
C	4.4	1.9	1.8	9.4	1.6	7.5	1.5	1.1	1.1	1.1	1.3	1.6	0.9
D	3.2	1.7	1.3	2.2	1.0	—	—	—	—	—	1.4	1.7	1.0
E	3.1	1.7	1.5	2.0	2.5	—	—	—	—	—	1.3	2.0	0.8
F	7.0	1.7	1.4	6.4	—	1.3	0.7	0.6	0.6	0.8	1.2	1.4	1.3
G control	3.4	2.5	2.0	1.2	2.5	1.2	1.0	—	—	—	1.0	1.1	1.0
H control	6.2	2.9	1.6	6.0	2.7	—	1.6	—	—	—	1.3	1.6	1.0
Q control	—	—	—	—	—	—	0.9	0.9	0.9	1.0	—	—	—

* $F(ab')_2$ fragments (0.2 μg) of sexually active homosexual patients and control subjects (G, H, Q) were adsorbed to microtiter plates that were then reacted with purified IgG of patients or control subjects at 12 serial dilutions of IgG, starting at 0.2 μg. ^{125}I-protein A was then added and assayed for radioactivity. The cpm obtained for each serial dilution was divided by the cpm obtained from a simultaneously run control experiment in which control $F(ab')_2$ fragments were reacted with autologous control IgG at serial dilution. The numbers given refer to the mean of the highest ratios obtained for a minimum of two consecutive dilutions. A ratio >1.3 is considered positive. It was determined from the 2 SD measurement obtained from six replicate determinations with six different control subjects. Each ratio is the mean of 2–5 experiments. $F(ab')_2$ fragments were passed through a protein A column and shown to be free of IgG as determined by both SDS-PAGE and radioimmunoassay. —, not done.

*Table III. Binding Ratios of AIDS HSITP Patient's IgG to F(ab')$_2$ Fragments of Patients and Controls Compared to Binding of Control IgG to Control F(ab')$_2$ Fragments**

F(ab')$_2$ fragments	Purified IgG immunoglobulin of AIDS patients with ITP			
	II	FF	GG	HH
FF	1.7 (2.5)	0.8	1.0	0.8
GG	2.8 (6.4)	1.0	1.1	1.0
HH	2.7 (4.5)	0.9	1.2	1.1
X	2.5 (5.4)			
G control	4.2			
H control	4.2			

* Methodology same as in Table 2. Patient II is a homosexual with *Pneumocystis carinii* pneumonia; patients FF, GG, and HH are homosexuals with Kaposi's sarcoma and ITP; patient X has NITP; subjects G and H are controls. Numbers in parentheses refer to ratios obtained with affinity-purified anti-F(ab')$_2$ obtained from the dissociated immune complexes of patient II absorbed to solid-phase F(ab')$_2$ of GG.

eluate had greater binding than the patient's serum for F(ab')$_2$ fragments FF, GG, HH, and X.

Anti-F(ab')$_2$ IgG antibodies in NITP patients. When purified IgG fractions of five NITP patients were tested against a panel of F(ab')$_2$ fragments from various patients and controls, all five reacted, but with limited reactivity, particularly against F(ab')$_2$ fragments of patients A and F (Table IV). Patient DD's purified serum IgG was affinity-purified on solid-phase F(ab')$_2$ from patient A. The reactivity of the highly purified anti-F(ab')$_2$ IgG increased its ratio to 9.0. This affinity-purified antibody was shown to have both kappa and lambda light chains. A sixth patient, JJ, had broad reactivity against the panel of 6 F(ab')$_2$ fragments of Table III, with ratios ranging from 2.2 to 2.9 (data not shown).

Absence of anti-F(ab')$_2$ IgG antibodies in ATP patients. In contrast, when the IgG fractions of six thrombocytopenic ATP patients were tested against the same panel of F(ab')$_2$ fragments as in Table I, no reactivity was noted. Reactivity was not noted when the IgG fractions of four ATP patients were tested against a panel of F(ab')$_2$ fragments from seven ATP patients (Table V).

Correlation of anti-F(ab')$_2$ IgG antibodies with presence of immune complexes in serum. 18 HSITP and NITP patients had circulating immune complex measurements performed on sera that averaged 2.16 mg/ml±0.48 (SEM), or 10-fold the value noted in healthy control subjects (0.21±0.20) (4). These were tested for anti-F(ab')$_2$ IgG reactivity. The binding ratios for specific IgGs with broad specificity against F(ab')$_2$ fragments were averaged to obtain a mean reactivity index. Mean reactivity index correlated with serum immune complex level, $r = 0.83$, $P < 0.01$, $n = 16$, using Spearman's rank correlation coefficient test.

Specificity of anti-F(ab')$_2$ IgG antibodies. The broad specificity of anti-F(ab')$_2$ Ig antibodies, noted particularly in homosexual patients, suggested that these were not antiidiotype antibodies. To confirm this suspicion, experiments were performed with two different purified multiple myeloma monoclonal F(ab')$_2$ fragments. Anti-F(ab')$_2$ IgG from patients A and II reacted equally well with both monoclonal F(ab')$_2$ fragments. Anti-F(ab')$_2$ Ig from patient A reacted equally well with both monoclonal F(ab')$_2$ fragments as well as F(ab')$_2$ fragments from patients A and B as well as control H (Table VI). These data indicate lack of idiotype specificity of anti-F(ab')$_2$ antibodies for F(ab')$_2$ fragments.

*Table IV. Binding Ratios of Narcotic Addict IgG to F(ab')$_2$ Fragments of Patients and Control Individuals Compared to Binding of Control IgG to Control F(ab')$_2$ Fragments**

F(ab')$_2$ fragments	Purified IgG immunoglobulin of narcotic addicts				
	AA	BB	CC	DD	EE
A	4.9	4.8	4.9	5.4 (9.0)‡	6.0
Z	0.8	0.8	0.8	0.7	0.8
V	1.5	1.0	1.0	1.1	1.1
E	1.0	1.0	1.0	1.2	2.1
F	2.4	2.0	1.8	1.6	1.8
W	1.0	1.0	1.2	1.5	1.2
X	0.9	0.8	0.8	0.8	0.8
H Control	1.4	1.1	0.9	1.0	1.0
Q Control	0.9	1.0	—	0.9	0.9

* Methodology same as in Table II. Patients A, Z, V have Kaposi's sarcoma and ITP; patients E and F have Kaposi's sarcoma, patients W and X have narcotic addict ITP, subjects H and Q are controls.
‡ When adsorbed to solid-phase F(ab')$_2$ of patient A and eluted, the ratio was 9.0.

Discussion

These data indicate the presence of high titer IgG anti-F(ab')$_2$ antibodies in the sera and immune complexes of thrombocytopenic patients at risk for AIDS, or with AIDS, that correlates

*Table V. Binding Ratios of ATP IgG to F(ab')$_2$ Fragments of HSITP Patients, Controls, and ATP Patients Compared to Binding of Control IgG to Control F(ab')$_2$ Fragments**

F(ab')$_2$ fragments	Purified IgG immunoglobulin of ATP patients						
Homosexuals	J	K	L	M	N	O	P
A	0.8	0.9	0.9	0.9	1.0	0.9	—
B	0.9	1.1	0.7	0.7	1.1	0.8	—
C	1.1	1.3	0.7	1.3	1.1	0.8	—
D	0.9	0.9	0.9	1.0	0.9	0.8	—
E	1.2	1.1	1.2	1.0	1.0	1.2	—
F	1.1	1.0	0.9	1.0	0.9	1.0	—
G control	0.8	0.9	0.7	0.9	1.0	0.7	—
ATP Patients							
J			0.8	0.9	—	1.1	0.9
K			1.2	1.1	—	1.2	1.6
L			1.0	1.1	—	0.8	0.9
M			0.9	0.9	—	0.8	0.9
N			1.1	1.0	—	1.0	0.9
O			1.0	1.1	—	0.8	1.0
P			1.0	1.5	—	1.1	1.0

* Methodology same as Table II. Patients A–F are sexually active homosexuals. Patients J–P are female patients with classic ATP. Subject G is a control.

Table VI. Binding Ratios of Homosexual Patients' IgG to F(ab')$_2$ Fragments of Monoclonal IgG of Multiple Myeloma Patients, Compared to Binding of Control IgG to Control F(ab')$_2$ Fragments *

	Purified IgG immunoglobulin of homosexual patients	
F(ab')$_2$ fragments	A	II
MIgG 1 (kappa)	2.4	2.9
MIgG 3 (kappa)	2.2	2.1
A	2.2	
B	2.5	
H control	2.8	

* MIgG 1 and MIgG 3 refer to F(ab')$_2$ fragments of purified monoclonal IgG obtained from two patients with multiple myeloma. A and B are patients with HSITP; II is a homosexual patient with Kaposi's sarcoma and ITP; H is a control subject. The ratios refer to the means of 2–5 experiments.

with the concentration of circulating immune complexes (data determined by the PEG method). This observation was suggested from preliminary studies on immune complexes in these patients that revealed specific antibodies against a host of viral antigens, with absence of the specific antigens. In contrast, patients with classic autoimmune thrombocytopenia have neither circulating immune complexes detected by this method, nor anti-F(ab')$_2$ antibodies. However, similar anti-F(ab')$_2$ antibodies have recently been reported in the sera of patients with rheumatoid arthritis (12–15) and systemic lupus erythematosus (12, 15–17), disorders of immune regulation that are associated with the presence of circulating immune complexes as well as autoantibodies.

The markedly elevated platelet-bound IgG, IgM, and C3C4 in these HSITP or NITP patients (2, 4) has suggested a role for immune complexes in the induction of thrombocytopenia. It is likely that some of the platelet-bound IgG and C3C4 represent immune complexes and that some of the immune complexes may be composed of IgG anti-F(ab')$_2$ complexes. Indeed, anti-Fab' antibodies have recently been demonstrated in the circulating immune complexes of patients with rheumatoid arthritis (18), and anti-F(ab')$_2$ antibodies in individuals immunized against keyhole limpet hemocyanin (KLH) (19).

The anti-IgG antibody found in these patients was not rheumatoid factor (IgM against IgG-Fc), nor IgM anti-IgG, but rather IgG anti-F(ab')$_2$. The reactivity for F(ab')$_2$ was broad with reactivity against control F(ab')$_2$ as well as autologous or homologous F(ab')$_2$ fragments for most of the homosexual patients studied. However, 3 of 12 HSITP and 5 of 6 NITP patients appeared to have limited reactivity against specific F(ab')$_2$ fragments. This suggested the possibility that some of these anti-F(ab')$_2$ antibodies might be antiidiotypes. Attempts to demonstrate specificity were unsuccessful, since F(ab')$_2$ fragments from two different purified monoclonal IgG preparations of multiple myeloma patients reacted equally well with different positive reacting anti-F(ab')$_2$ preparations. Nevertheless, since affinity-purified anti-F(ab')$_2$ antibodies were polyclonal, (kappa and lambda light chains), the possibility of specific antiidiotype antibody has not been ruled out. For example, if anti-AIDS–associated retrovirus antibody can be affinity purified, it would be of interest to determine whether anti-F(ab')$_2$ antibodies could block binding of anti-AIDS–associated retrovirus antibody to its epitopes on the virus. Indeed, IgG anti-F(ab')$_2$ antibodies with blocking activity against anti-DNA antibody (16, 17), as well as antitetanus toxoid antibody (17) have recently been reported in patients with SLE; whereas anti-F(ab')$_2$ antibodies of patients with rheumatoid arthritis did not block anti-DNA antibody of SLE patients (16). In addition, individuals immunized with KLH have anti-F(ab')$_2$ antibodies against anti-KLH antibody in their circulating immune complexes (19). The etiology of the circulating immune complexes as well as the autoimmune and alloimmune anti-F(ab')$_2$ antibodies in these patients at risk for AIDS, or with AIDS, is not readily apparent. Experimental anti-antibodies have been produced after immunization of rabbits with antigen as well as antigen-antibody complexes, however (20–22). It has been postulated that immune complex formation results in unfolding of sites on the autologous IgG molecule that could expose buried antigenic determinants not recognized by the host (20). Indeed, human IgG anti-F(ab')$_2$ or anti-IgG antibodies have been noted in normal subjects (23, 24) that could be adsorbed out or dissociated with immune complexes (23, 24), or F(ab')$_2$ fragments (23), but not with intact IgG (23). Repeated antigenic stimulation in patients at risk for AIDS could lead to the very high level of circulating immune complexes noted, which in turn may contribute to the formation of anti-F(ab')$_2$ antibodies, thus perpetuating immune complex formation. It is possible that the circulating immune complexes and anti-F(ab')$_2$ result from exposure to the AIDS-associated retrovirus as well as other viruses. In addition, since AIDS is a disorder of immune regulation, it is likely that these anti-F(ab')$_2$ antibodies, which are present at low levels in some normal control subjects (12, 16, 23), have lost their immunoregulatory control mechanisms. A disorder of down regulation of anti-F(ab')$_2$ antibody production thus might reflect perturbation of the T cell-B cell network by the AIDS-associated retrovirus. It is possible that some, if not all, of the postulated mechanisms are responsible for the elevated circulating immune complexes and anti-F(ab')$_2$ antibodies in patients at risk for AIDS, or with AIDS.

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