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IgG subclass response to HIV in relation to antibody-dependent cellular cytotoxicity at different clinical stages

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SUMMARY

The anti-HIV IgG subclass response was analysed in sera from different clinical stages and related to virus specific antibody-dependent cellular cytotoxicity (ADCC). IgG1 was found to be the dominant subclass, present in all sera and with similar mean titres at different stages. The number of anti-HIV IgG3 positive sera, measured on whole viral lysate antigen plates, decreased during disease progression from 38% in symptom-free to 7% in AIDS patients. IgG2 and IgG4 subclasses were less prevalent although a slight increase of IgG4 frequency was found in AIDS patients. High IgG1 titres correlated with a positive ADCC reaction but there was no correlation between anti-HIV IgG1 and ADCC titres. Some sera which contained HIV IgG1 as the only subclass were able to mediate an ADCC reaction. In addition, when anti-HIV IgG3 was isolated, by protein A chromatography, no ADCC killing was induced by these antibodies. It is concluded that IgG1 is the major ADCC-active IgG subclass in HIV infected individuals. The lack of correlation between IgG1 and ADCC titres may be explained by a relatively small fraction of IgG1 antibodies mediating ADCC.

Keywords IgG subclass HIV ADCC AIDS

INTRODUCTION

HIV (human immunodeficiency virus)-infection may develop from an asymptomatic stage through persistent generalized lymphadenopathy (PGL) to full-blown AIDS (Seligmann *et al.*, 1987). HIV infects both CD4 positive helper T cells and cells within the reticuloendothelial system (Seligmann *et al.*, 1987). The infection causes a profound T cell defect and a polyclonal B cell activation (Aucouturier *et al.*, 1986; Seligmann *et al.*, 1984; El-Sadr *et al.*, 1984; Zollar-Pazner, 1984; Amman *et al.*, 1982; Stahl *et al.*, 1982; Lane *et al.*, 1983; Chess et al., 1984). In some infected individuals, the immune system successfully controls HIV expression during long asymptomatic periods. Consequently, it is important to identify immune parameters which participate in the elimination of infected cells. Antibody-dependent cellular cytotoxicity (ADCC) is believed to be one such parameter (Wigzell, 1985). High-titred, HIV-specific ADCC can be detected in sera from all clinical stages (Rook *et al.*, 1987; Ljunggren *et al.*, 1987a).

Virus specific IgG1 and IgG3 are the dominant IgG subclasses formed in response to viral antigens, where an increase of IgG3 may indicate viral disease (Linde, 1985). IgG2 appears to be predominantly reactive with carbohydrate antigens (Riesen, Skvarel & Braun, 1976). An increase of virus-specific IgG4 may reflect repeated stimulations with viral antigens (Linde, 1985). Both IgG1 and IgG3 mediate important protective, biological functions such as complement fixation, opsonization and induction of ADCC by NK cells (Schumacher *et al.*, 1976; Anderson & Looney, 1986).

Increased total IgG1 and IgG3 levels and decreased IgG2 and IgG4 levels have been reported in HIV infections (Aucouturier *et al.*, 1986). Those changes have been postulated to be caused by a combination of unspecific, polyclonal B cell activation and impaired helper T cell activity (Aucouturier *et al.*, 1986). Also, a restricted HIV-specific IgG subclass response has been reported with a decrease of all subclasses except IgG1 during disease progression (Sundqvist *et al.*, 1986).

In the present work we have measured HIV-specific IgG subclasses in different clinical stages in relation to ADCC-active antibodies which mediate killing of HIV infected target cells.

MATERIALS AND METHODS

Cell lines

Cells were propagated in RPMI-1640 medium with 10% fetal calf serum (GIBCO, Grand Islands, NY) and antibiotics. They were subcultured twice weekly. The U937 cell line is derived from a histiocytic lymphoma (Sundström & Nilsson, 1976). Phenotypically stable subclones have been isolated from the

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Table 1. Distribution of HIV-specific IgG subclasses within patient categories

Clinical evaluation	No. of patients	HIV-specific subclass response Frequency positive, median titre and range of positive sera IgG1	IgG2	IgG3	IgG4
Symptom-free	34	100%	18%	38%	12%
		25,000	1,700	90	300
		(100–100,000)	(430–9,000)	(50–1,000)	(50–400)
PGL*	35	100%	6%	22%	17%
		38,000	350	90	500
		(300–100,000)	(100–600)	(50–3,000)	(100–700)
ARC†	18	100%	11%	11%	11%
		30,000	325	350	100
		(700–100,000)	(50–600)	(300–400)	(100–100)
AIDS	15	100%	7%	7%	27%
		47,000	640	780	2000
		(1,000–100,000)			(1,000–5,000)

* Persistent generalized lymphadenopathy.
† AIDS related complex.

parental U937 cell line (Åsjö *et al.*, 1987). For the present experiments, clone 2 was infected with HTLV-III B and kept in continuous passage. Eighty to ninety-five per cent of the cells were HIV-positive as determined by indirect immunofluorescence on fixed cells (Ljunggren et al., 1987b).

Sera

Sera were obtained from uninfected controls and from HIV infected individuals, either asymptomatic or with clinical symptoms of persistent generalized lymphadenopathy (PGL), AIDS related complex (ARC) or AIDS. All sera were tested for HIV antibodies by an enzyme-linked immunosorbent assay (ELISA, Organon Teknica, Oss, The Netherlands) and confirmed by Western blot strips (DuPont, Pharmaceuticals, Wilmington, Del.).

Isolation of lymphocytes

Peripheral blood lymphocytes were obtained by density gradient centrifugation on Ficoll-Isopaque (Pharmacia, Uppsala, Sweden) and washed three times in phosphate-buffered saline. Cells were then resuspended in RPMI-1640 containing 10% fetal calf serum. Adherent cells were removed by the scrubbed nylon wool column technique (Merrill, Ullberg & Jondal, 1984). The isolated effector cells consisted of non-adherent T cells and null cells.

^{51}Cr-release cytotoxicity test

Cytotoxocity assays were performed in duplicate wells in v-shaped microplates using a total volume of 150 μl. ^{51}Cr labelled HTLVIII B infected U937, clone 2, cells (1×10^4) and separated lymphocytes from non-infected individuals (2×10^5) were added together with 50 μl of serum dilutions. Fifty microlitres of the supernatant from each well was harvested after 3 hours and counted in a gamma counter. Released radioactivity was calculated as described (Merrill, Ullberg & Jondal 1984). HIV specific ADCC was determined as follows:

Specific ^{51}Cr release with HIV-positive sera − Specific ^{51}Cr release with HIV-negative sera.

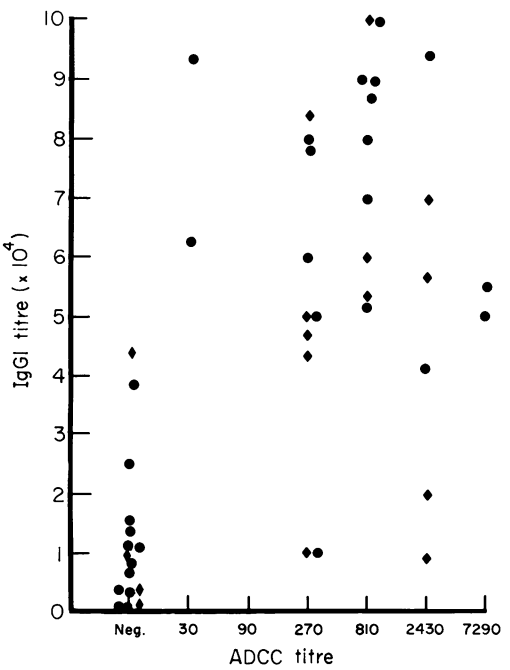

Fig. 1. Forty-six sera were analysed for HIV-specific ADCC- and IgG1 titres. (♦) Sera with reactivity to IgG1 only. (●) Sera with more than one subclass present.

No serum could induce ADCC against the uninfected target cell and none of 40 HIV-antibody negative sera could induce ADCC against the infected or uninfected target cells. No killing was induced with sera without effector cells. The ^{51}Cr-spontaneous release did not exceed 10%.

HIV-specific ADCC index, SAI (Ljunggren *et al.*, 1987b), was calculated as a ratio:

$$\frac{\text{HIV specific ADCC}/\beta_2 \text{ microglobulin specific ADCC}}{\text{\% of infected target cells}} \times 100$$

Sera with SAI $>0{\cdot}5$ at 1:30 dilution were considered to be positive for HIV specific ADCC.

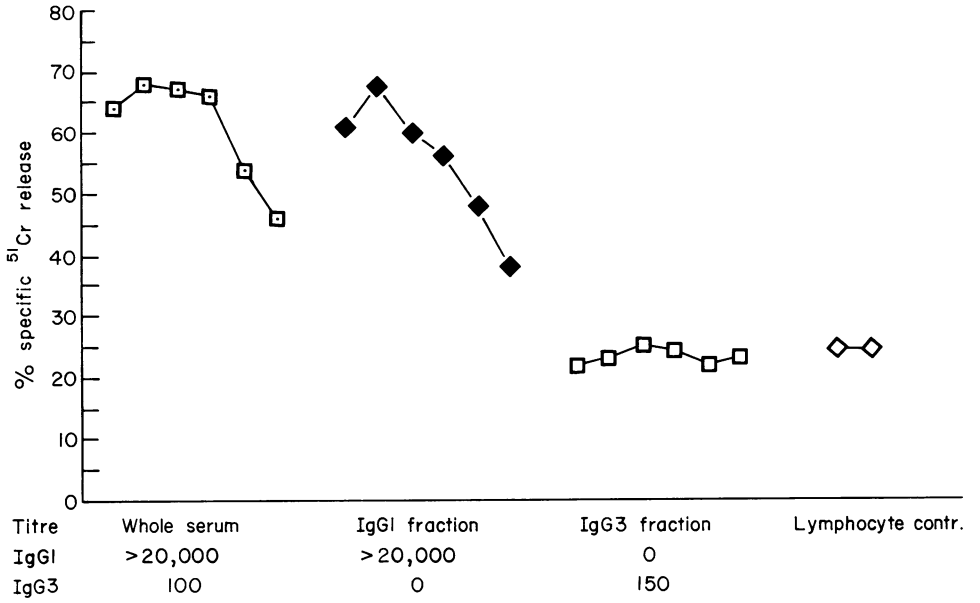

Fig. 2. ADCC and ELISA titres in IgG1 and IgG3 subclass fractions. The effector : target cell ratio was 20 : 1. The serum dilutions were 1 : 30–1 : 7,290 (6 consecutive steps, 1 : 3 dilutions).

Table 2. Relation of ADCC reactivity to clinical stages and to HIV IgG1 litres

		IgG1 anti-HIV median titre (*n*)			
Clinical evaluation	No. of patients	Sera	ADCC-positive	Sera	ADCC-negative
Symptom-free	14	78,000	(7)	5,000	(7)
PGL	14	63,000	(8)	14,500	(6)
ARC	2	62,000	(1)	43,000	(1)
AIDS	9	60,000	(9)	—	(0)
Unknown	7	43,000	(5)	1,500	(2)

Subclass ELISA

HIV-specific IgG subclasses were determined on the Organon Teknica HIV plates. Serum samples were diluted in 10-fold steps, 1 : 10–1 : 100,000, in Organon dilution buffer with fetal goat serum. One-hundred microlitres of the dilutions were added to each well, and incubated for 120 min at 37°C. After washing, 100 μl monoclonal antibodies used in the following dilutions were added for 105 min at 37°C: For IgG1, clone BAM 15 1 : 2000, for IgG3, clone BAM 8 1 : 2000, for IgG4, clone BAM 11 1 : 800 (Seward Laboratories, London), and for IgG2, clone HP 6002 1 : 100 (Dr. C. Reimers, Center for Disease Control, Atlanta). The plates were washed and 100 μl of peroxidase-conjugated rabbit anti-mouse IgG (DAKO Patts, Copenhagen, Denmark) was added for 105 minutes at 37°C. The substrate ortho-phenylendiamine, activated by H_2O_2, was added. The reaction was terminated after 30 min at room temperature by adding 100 μl 2·5 M H_2SO_4. The absorbance was measured at 490 nm. The background levels were taken as the mean + 3 s.d. absorbance values with HIV antibody negative sera.

Protein A Sepharose affinity chromatography

Protein A Sepharose CL 4B (Pharmacia Fine Chemicals AB, Uppsala, Sweden) was packed in a final bed volume of 7 ml. Human IgG1, IgG2 and IgG4 are adsorbed to Protein A, while IgG3 is not (Kronwall et al., 1969). The sera were added to the column with PBS pH 7.4 as starting buffer, and a flow rate of 19 ml/cm^{-2}/h. Adsorbed molecules were eluted with 0·1 M glycin-HCl pH 2·8. The eluate was kept on ice and immediately neutralized to pH 7·5 by solid tris.

Statistical analysis

When comparing differences in ADCC titres Student's *t*-test combined with a Mann–Whitney U-test was used.

RESULTS

Subclass distribution in HIV-infected patients

The subclass distribution of HIV-specific IgG is shown in Table 1. All patients had HIV IgG1, often in high titres. The distribution of titres and the median titres were similar in the various stages. The other subclasses occurred in varying frequencies, in considerably lower titres than HIV IgG1. The frequency of HIV IgG3 positive sera appeared to decrease with advancing stages. All IgG3 antibodies may not have been detected by the subclass ELISA using Organon plates, since IgG3 anti-HIV is, to a large extent, directed to HIV p17 (Klasse and Blomberg, 1987; Broliden *et al.*, submitted). This antigen may be under-represented in the Organon plates. HIV IgG4 was most frequent in AIDS patients.

The number of different HIV subclasses in individual patients varied with stage of disease. A more restricted pattern with IgG1 as the single HIV IgG developed in 29/33 (88%) of patients with ARC and AIDS as opposed to 26/89 (29%) of symptom-free patients or patients with PGL. In 50 healthy control persons no anti-HIV IgG subclasses were found.

ADCC properties related to HIV IgG subclasses

Forty-six sera were selected from the samples in Table 1 to represent various stages of disease and subclass IgG patterns.

Sera with no detectable ADCC activity had lower levels of IgG1 than sera with ADCC activity. The median IgG1 ELISA titres were 9,000 (range 100–45,000, $n=16$) and 60,000 (range 9,000–100,000, $n=30$), in negative and positive sera respectively (Fig. 1). Thirteen of 16 (81%) ADCC negative sera but only three of 30 (10%) ADCC positive sera had IgG1 titres below 1 : 15,000 ($P<0{\cdot}001$). Within the ADCC positive group, no clear relation was seen between ADCC titres and HIV IgG1 titres.

Twelve of 30 ADCC positive sera from different clinical stages had IgG1 as the only detectable subclass (Fig. 1). IgG1 titres of these twelve sera ranged from 8,000–100,000. These sera could still mediate ADCC to the same extent as sera with one or more of the other subclasses present. The mean value of ADCC titres in sera with only IgG1 was 1125 ($n=12$) and in sera with additional subclasses 1473 ($n=18$) ($P>0{\cdot}05$).

Since IgG1 was shown to mediate HIV specific ADCC, the ability of IgG3 was also examined. Three sera which mediated high-titred (> 1 : 7,000) HIV specific ADCC and contained both HIV specific IgG1 and IgG3 were selected for IgG3 separation. IgG1, IgG2 and IgG4 subclasses were adsorbed with Protein A Sepharose affinity chromotography while IgG3 passed. The adsorbed subclasses were then eluted. Both fractions were tested for subclass reactivity with ELISA. In Fig. 2, the ability to mediate ADCC is shown for whole sera, for the eluted material and for the IgG3 fraction. IgG1 was the only detectable subclass in the eluted material and showed similar ADCC activity as whole sera. The IgG3 fraction, with no other subclasses present, lacked ability to mediate ADCC.

Only two sera had IgG2, one ADCC negative and one positive. The median IgG3 ELISA titres for the ADCC negative and positive sera were 50 ($n=8$) and 30 ($n=8$) respectively. Eight of 10 sera which contained HIV IgG4 reactivity were ADCC positive (data not shown).

Clinical stages of patients were related to ADCC reactivity and HIV IgG1 titres (Table 2). ADCC reactivity occurred in all stages. Here too, ADCC reactivity appeared related to HIV IgG1 titres. There are however some sera (four of 17 sera with HIV IgG > 20,000), (Fig. 2) with high HIV IgG1 titre and poor ADCC reactivity.

DISCUSSION

IgG1 appears to be the predominant virus-specific IgG subclass in HIV infected individuals. This has also been found in other viral systems (Linde, 1986). HIV IgG1 was present in all sera tested ($n=102$), with similar median titres at different clinical stages. A restricted pattern with IgG1 as the single HIV-IgG subclass developed in later disease stages. HIV IgG2-4 showed lower median titres and were less prevalent than IgG1. About 20% of asymptomatic patients had IgG2 and there was a tendency for them to decrease (18%–7%) during disease progression as reported earlier (Sundqvist *et al.*, 1986). IgG3 frequency decreased from 38% in symptom-free subjects to 7% in AIDS patients. Increases of total IgG1 and IgG3 antibodies are considered to be caused by an unspecific polyclonal B cell activation (Aucouturier *et al.*, 1986). Our results demonstrate that in many patients there is an increase in titres of specific IgG1 antibodies, but not in the frequency of other subclasses. HIV IgG4, on the other hand, was most frequent in AIDS sera. This is in accordance with previous findings of Herpes Simplex virus (HSV)-specific IgG4 which increases after repeated recurrences of HSV-infection (Sundqvist, Linde & Wahren 1984). The alterations in IgG2-4 subclass levels may be useful markers for disease progression.

High HIV IgG1 titres were found to correlate with presence of HIV-specific ADCC, although there was no correlation with ADCC titres. Some sera, with IgG1 as the only detectable subclass, could mediate ADCC. It is concluded that IgG1 is the major ADCC active IgG subclass in HIV infected individuals. IgG1 anti-HSV has been demonstrated to mediate ADCC (Mathiesen *et al.*, 1988). IgG1 and IgG3 are known to interact with Fc receptors expressed on ADCC effector cells, and to trigger killing (Anderson & Looney, 1986; Sunada, Suzuki and Ota, 1985; Urbaniak & Greiss, 1980; Goff *et al.*, 1984).

The ADCC active antibodies are predominantly directed against envelope antigens (Ljunggren *et al.*, 1988). It cannot be excluded that other viral antigens also expressed on the infected host cells, may serve as target determinants. Hendry *et al.* (1987) analysed the antigenic specificity of anti-HIV antibodies and found increased levels of both IgG1 and IgG2 antibodies to pol and gag antigens in early disease. They found similar levels of anti-envelope antibodies in all disease stages. We found that median levels of HIV specific IgG1 do not vary at differerent disease stages. It is therefore unclear why AIDS patients have decreased ADCC titres since such antibodies are predominantly of the IgG1 subclass. This may be explained by the fact that the relative amount of ADCC mediating antibodies is so small that a drop is not reflected in the whole specific IgG1 fraction. Viral IgG3 has revealed neutralizing and complement dependent properties unique to this subclass (Mathiesen *et al.*, 1988). IgG3 was therefore separated from IgG1 but did not give an ADCC reactivity of its own. Also, HIV specific IgG3 is mainly directed against gag proteins (Klasse & Blomberg, 1987; Broliden *et al.*, submitted). Contribution of HIV IgG3 to ADCC therefore seems less likely.

Only populations of anti-envelope IgG1 antibodies which react with selected determinants within the gp120/41 glycoproteins might induce ADCC. The CD4 receptor site, the cleavage region between gp120 and gp41 and some hydrophobic regions of gp41 are conserved (Starcich *et al.*, 1986; Myers *et al.*, 1987). Antibodies against these regions are likely to be group-specific and reactive with all HIV-1 strains and thus independent of any genetic drift in the viral genome. Such antibodies might be protective if they can mediate functions such as ADCC or virus neutralization. For the detection of antibodies against defined regions, site directed serology can be used with representative peptides (Norrby *et al.*, 1987). In an extension of the present work we will investigate IgG subclass reactivity with defined peptides, representing conserved regions, and analyse their functional characteristics.

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