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Isotypy of serum monoclonal immunoglobulins in human immunodeficiency virus-infected adults

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SUMMARY

The classes, subclasses and light chain types of 78 serum monoclonal immunoglobulins (MoIg) from adult patients affected with various clinical forms of human immunodeficiency virus (HIV) infection were studied by a sensitive Western blot technique. The incidence of MoIg-containing sera was 26% in a systematic study. Most of these sera contained several (up to eight) detectable MoIg. These MoIg were IgG (91%) and IgM (9%) with a predominance of light chains of the λ type ($\kappa:\lambda$ ratio 0·6). The subclass distribution of monoclonal IgG was strikingly different from that observed in myeloma; much less IgG1 and much more IgG3 and IgG4.

Keywords HIV AIDS immunoglobulin subclasses monoclonal immunoglobulins

INTRODUCTION

Polyclonal hyper-immunoglobulinemia is a common finding in patients affected with the various symptomatic forms of human immunodeficiency virus (HIV) infection (Seligmann *et al.*, 1984). More recently, using the immunofixation technique, Papadopoulos *et al.* (1985) reported a high incidence of monoclonal or oligoclonal immunoglobulins (Ig) in acquired immunodeficiency syndrome (AIDS) patients affected with Kaposi's sarcoma (KS). Subsequently, the presence of serum monoclonal Ig (MoIg) was also documented using the same method in asymptomatic seropositive subjects and in patients affected with the lymphadenopathy syndrome (LAS) (Heriot, Hallquist & Tomar, 1985; Bouscary *et al.*, 1987; Lefrere *et al.*, 1987; Papadopoulos & Costello, 1987; Sala *et al.*, 1987). None of these studies includes a determination of the subclasses of MoIg and some of them deal with short series. We report here on the characterization by a Western blot technique of the classes, subclasses and light chain types of a number of MoIg in the serum of HIV-infected patients. We found an isotypic distribution of MoIg strikingly different from that observed in other conditions.

MATERIALS AND METHODS

Patients

This study includes a systematic study of consecutive sera from 62 HIV-infected subjects (one healthy subject, 31 patients with LAS, eight with AIDS-related complex (ARC) and 22 with AIDS). In addition, eight sera (five from LAS, two from ARC and one from AIDS patients) were previously known to contain MoIg by immunofixation. All patients were infected with HIV-1 but for an LAS patient in whom both HIV-1 and HIV-2 infections were evidenced. As for the AIDS patients, 18 had opportunistic infections (OI), two KS, two both OI and KS and one Burkitt's lymphoma (BL). These patients belonged to the various known risk groups with a distribution analogous to that known in the whole country.

Methods

Electrophoretic and immunoelectrophoretic (Iel) analysis was performed according to usual methods. MoIg were characterized by an immunoblotting technique (Aucouturier *et al.*, 1987, Aucouturier & Preud'homme, 1987). Briefly, sera diluted 1/50 to 1/1000 were separated by thin layer agarose electrophoresis (Paragon, Beckman, Gagny, France) and transferred to nitrocellulose sheets by pressure blotting. After saturation for 1 h with 5% powdered skimmed milk, the blots were revealed with peroxidase- or alkaline phosphatase-coupled antibodies specific for human γ, α, μ, κ and λ chains (Diagnostics Pasteur, Paris, France) at the appropriate dilutions in 0·01 M phosphate 0·15 M NaCl pH 7·4 buffer containing 2% bovine serum albumin, or with anti-IgG subclass monoclonal antibodies (NL16 at 1/200 for IgG1, a mixture of GOM2, 1/100 and HP 6014, 1/100 for IgG2, ZG4, 1/200 for IgG3 and RJ4, 1/200 for IgG4, respectively) (Unipath, Bedford, UK) followed by peroxidase-coupled anti-mouse IgG antibodies (extensively absorbed on human IgG, prepared in our laboratory). The specificity and working dilutions of these various reagents were determined using the same immunoblotting procedure with myeloma sera and urines containing known MoIg. This method allows us to detect MoIg up to concentrations as low as 25 ng/ml, it is cheaper and more

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Table 1. Incidence of MoIg-containing sera according to the clinical groups (systematic study*)

	number of sera	
clinical group	studied	with MoIg
asymptomatic infection	1	1
LAS	31	7 (23%)
ARC	8	1
AIDS	22	7 (32%)
Total	62	16 (26%)

* additional sera with known MoIg were collected in 5 LAS, 2 ARC and 1 AIDS patients.

Table 2. Distribution of the sera according to the number of detectable MoIg by serum

	Number of MoIg/serum							
	0	1	2	3	4	5	6	8
number of sera	46	4	5	6	3	4	1	1
% of MoIg-containing sera	/	17	21	25	12	17	4	4

accurate than immunofixation, as shown by a comparative study of the same sera (Aucouturier *et al.*, 1987), with the advantage of the use of antisera of controlled specificity.

RESULTS

As shown in Table 1, MoIg were detectable by immunoblotting (and not by Iel analysis which showed only polyclonal hyper-immunoglobulinemia) in all studied clinical groups and about one patient out of four had serum MoIg. Most of these sera contained several (up to eight) identifiable MoIg (Table 2). All AIDS patients whose sera contained MoIg had OI and none of them had KS nor BL. The number and isotypic distribution of MoIg did not significantly differ between clinical groups. A total of 70 MoIg were characterized in 24 sera (16 from the random study and eight previously known to contain MoIg). They were IgG (91%) or IgM (9%). Strikingly, 43 IgG and six IgM bore λ light chains versus only 28 IgG κ and one IgM κ ($\kappa:\lambda$ ration in the whole series: 0·6). The subclass distribution of monoclonal IgG was clearly different from that observed in multiple myeloma with much more IgG3 and IgG4 and less IgG1 in HIV-infected patients (Table 3). The majority of sera without detectable MoIg showed a restriction of heterogeneity of polyclonal IgG, with a predominance of cathodic molecules.

Table 3. Subclass distribution (%) of serum monoclonal IgG in HIV infections and multiple myeloma

	IgG1	IgG2	IgG3	IgG4
HIV infections	42·5	19	23·5	15
myeloma*	76·1	14·2	3·4	6·3

* data from Aucouturier & Preud'homme (1987).

DISCUSSION

The present study confirms previous findings that serum MoIg undetectable by conventional Iel commonly occur in patients infected by HIV, with an incidence analogous to that previously reported in certain clinical groups (Papadopoulos *et al.*, 1985; Heriot *et al.*, 1985, Papadopoulos & Costello, 1987; Lefrere *et al.*, 1987; Bouscary *et al.*, 1987, Sala *et al.*, 1987), and extends this observation to other clinical groups, so that it is clear that MoIg may be observed in asymptomatic subjects and in patients with LAS, ARC, AIDS with OI or KS. We found no difference in isotypic distribution and incidence of MoIg according to clinical presentation. As in previous studies, MoIg were usually several. A cathodic restriction of polyclonal IgG, possibly reflecting subclass imbalances (Aucouturier *et al.*, 1986), was also common. In fact a serum pattern featuring several MoIg in small amounts and restricted IgG of slow mobility is of diagnostic value. It led us to search for HIV infection in some of the present patients in whom this possibility had not been considered before.

The isotypic distribution of MoIg in the present study, with their λ predominance, absence of monoclonal IgA and striking IgG subclass imbalance appears to be unique. The predominance of light chains belonging to the λ type was not reported before. Previous studies were performed using commercial immunofixation, which in our experience in HIV infection does not identify every MoIg recognized by Western blotting and is sometimes hampered by antibody specificity problems. Some of these studies deal with short series and the light chain type of MoIg is not always clearly mentioned. Control experiments and our experience in other conditions featuring small amounts of MoIg undetectable by Iel rule out the lack of monoclonal IgA to be due to a technical problem. We are presently studying pediatric AIDS and the single MoIg found in nine child sera was an IgA. As for IgG subclasses, they were not studied previously. Their distribution is very uncommon with a relatively low incidence of IgG1 and a high incidence of IgG3 (which outnumbered IgG2) and IgG4. This does not reflect the pattern of IgG subclass level imbalance found in LAS and AIDS. Indeed, in spite of important variations from patient to patient, mean IgG1 and IgG3 levels were high whereas IgG2 and IgG4 levels tended to be low (Aucouturier *et al.*, 1986). Subclass levels were measured in six sera with MoIg and there was no clear correlation with the isotypy of MoIg. This is not surprising in view of the small amounts of these MoIg.

The presence of serum MoIg in HIV-infected subjects indicates oligoclonal B cell expansions. They occur in asympto-

matic subjects and in patients with any clinical presentation. This is in agreement with the finding of multiple clonal B cell expansions by Ig gene rearrangement analysis in 20% of LAS patients (Pelicci *et al.*, 1986). Rearrangement or translocation of the c-myc oncogene were undetectable in the latter patients, in contrast to most cases of lymphomas occurring in AIDS (Groopman *et al.*, 1986; Pelicci *et al.*, 1986). It has been suggested that B cell clonal expansions may result from the combination of cellular immunodeficiency and stimulation by antigens and viruses. This possibility should lead to different incidences of MoIg according to the clinical groups, which is not the case. It is also worth noting that serum titres or antibodies to cytomegalovirus, Epstein–Barr virus and hepatitis B virus in patients with MoIg are unremarkable (Bouscary *et al.*, 1987). There is no evidence for a direct role of HIV in B cell expansion either (Pelicci *et al.*, 1986). Therefore, the high incidence of MoIg in HIV infection remains poorly explained. Whether the occurrence of MoIg has a predictive value with respect to the development of immunodeficiency or of lymphoma will probably be known from the follow-up of the patients in this and other series.

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