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Characterization and large production of human monoclonal antibodies against the HIV-1 envelope

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SUMMARY

Peripheral blood lymphocytes from a volunteer immunized with a recombinant vaccinia virus VSC-25 expressing the gp160 *env* protein of HTLV-IIIB strain and from an asymptomatic HIV-infected individual were immortalized by Epstein–Barr (EBV). Clones which secrete human monoclonal antibodies from the two individuals (DZ, IgG1, λ and C31, IgG1, κ) were obtained and were stable for more than 2 years. The two monoclonals were directed against the gp160 *env* protein of HIV, DZ directed against the gp41 and C31 directed against the gp120. C31 was group-specific, whereas DZ was directed against the HTLV-IIIB and HTLV-RF strains. The epitope recognized by DZ was mapped to the carboxy terminus of the gp41, by expression of HIV DNA fragments in a yeast system and peptide analysis. The C31 epitope was not expressed by the yeast library and not present among the peptides which were tested. Monoclonal antibodies had no inhibitory effect in an HIV-induced cell fusion assay, but DZ showed a weak neutralizing activity against the HTLV-IIIB strain. Cloned EBV-transformed cell lines were fused to a murine myeloma, which allowed the heteromyeloma to be cultivated in serum-free medium. The monoclonal antibodies were produced in large quantity in a hollow-fibre reactor at defined culture conditions and purification procedures.

Keywords human monoclonal antibodies HIV-1 envelope AIDS passive immunotherapy

INTRODUCTION

HIV infection usually induces a strong immune response that protects temporarily against disease progression (Weiss *et al.*, 1985; Robert-Guroff, Brown & Gallo, 1985; Ho, Rota & Hirsh, 1985). Indeed, high titre of neutralizing antibodies has been described in healthy HIV-infected individuals in contrast to AIDS patients (Ho *et al.*, 1987). In order to limit disease progression, passive immunization protocols have been developed (Karpas *et al.*, 1988; Jackson *et al.*, 1988). In response to this treatment, viral p24 antigen levels were decreased and antibody titres were maintained or increased. These passive immunizations were carried out with hyperimmune plasma collected from healthy infected individuals with high anti-HIV neutralizing antibody titres. The quality of this type of material is not constant and its quantity is restricted for ethical reasons. In contrast, human monoclonal antibodies (MoAbs) display many advantages; they are monospecific, have defined titres, and are permanently available.

Human MoAbs against gp120 and gp41 have already been produced by B lymphocytes from HIV-infected patients immortalized by Epstein–Barr virus (EBV) or by fusion with murine myeloma cells (Banapour *et al.*, 1987; Sugano *et al.*, 1988; Evans *et al.*, 1988; Grunow *et al.*, 1988; Desgranges *et al.*, 1988; Gorny *et al.*, 1989; Amadori *et al.*, 1989; Pollock *et al.*, 1989; Robinson *et al.*, 1990; Bugge *et al.*, 1990). Furthermore, *in vitro* primary immunization can be used to obtain human antibodies from B lymphocytes of non-infected individuals, as described by Ohlin *et al.* (1989). Recently, a chimeric mouse anti-human antibody directed against HIV-1 has been described (Liou *et al.*, 1989).

In view to test the feasibility of preparing human monoclonal antibodies for therapeutic purposes and an effective source of a large spectrum of neutralizing anti-HIV immunoglobulins, we established other cell lines producing antibodies. In this paper, we report the characterization of two human MoAbs, MoAbDZ and MoAbC31, obtained from a non-infected immunized volunteer, and from an HIV-infected asymptomatic individual, respectively. The two human monoclonal antibodies react with gp160, MoAbDZ directed against the transmembrane protein and MoAbC31 against the outer envelope protein.

In order to carry out pilot experiments for the production of human anti-HIV immunoglobulins in large volume for possible treatment of HIV infection, the two lymphoblastoid cell lines were fused to a murine myeloma, adapted to a serum-free

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medium and cultivated in a hollow-fibre reactor. After purification, these human MoAbs were not toxic and maintained their anti-HIV specificities.

MATERIALS AND METHODS

Lymphocytes and EBV transformation

Lymphocytes from an immunized volunteer were isolated 85 days after the first boost with recombinant vaccinia virus (VSC-25) infected fixed cells expressing the complete gp160 *env* protein of the HTLV-IIIB strain (Zagury *et al.*, 1988) and from an HIV-seropositive asymptomatic blood donor from the Centre Regional de Transfusion Sanguine de Lille, France.

Immortalization was performed by exposing lymphocytes to EBV supernatant from the B95-8 cell line. Cells were cultured at 1×10^6 cells/ml with cyclosporin A (0·1 μg/ml), in RPMI 1640 (Flow Laboratories, Irvine, UK) supplemented with 15% (v/v) heat-inactivated fetal calf serum (FCS) (Biosys, Compiegne, France) (Desgranges *et al.*, 1987).

After immortalization, B cell supernatants were tested for the presence of anti-HIV antibodies by immunofluorescence on fixed HIV-infected cells, ELISA and immunoblot. The cells were cloned by limiting dilution to 0·5 cell per well, on a feeder layer (10^5 cells per well) or irradiated human peripheral blood lymphocytes (25 Gy) and supernatants were screened again for the reactivity against HIV.

Fusion and cloning

Cloned EBV-transformed secreting cell lines (1×10^8 cells) were fused with murine myeloma P3X63Ag8.653 ($3{\cdot}3 \times 10^7$ cells) on a murine peritoneal macrophages feeder layer (2×10^3 cells/well). Plates were incubated under 7% CO_2 at 37°C and the medium was changed twice a week with DMEM (Eurobio, Paris, France), supplemented with 20% FCS (Anval, Rennes, France), HAT (100 μM hypoxanthine, 0·4 μM aminopterin and 16 μM thymidine), and ouabain 0·5 μM (Sigma Chemical Co., St Louis, MO).

High producing cells were then cloned by limiting dilution to 0·5 cells per well on a feeder layer of murine peritoneal macrophages.

Adaptation to serum-free culture

After fusion, the subclones were maintained in serum-free medium MS4 containing salts, glucose and glutamine according to DMEM formula; amino acids, vitamins and others constituants according to DMEM/F12 (1/1) formula; 20 μm ethanolamine; 50 μM 2-mercaptoethanol; 10 nM sodium selenite; 17 μM ascorbic acid, 0·5 g/*l* human fatty acid-free TNBP/Tween treated albumin (CRTS, Lille, France); 3·57 μM linoleic acid bound to human albumin; 3 μM reduced glutathion; and 0·5 mM ferric citrate.

Anti-HIV-specific antibody detection

Antibody screening was performed with Diagnostics Pasteur ELISA (Marnes la Coquette, France) and Wertern blot assays on anti-HIV-positive culture supernatants were carried out with commercial kits (Diagnostics Pasteur, and Dupont Biotechnology Systems Division, Wilmington, NY) according to manufacturer's instructions.

Indirect immunofluorescence (IIF) on aceton-fixed cells was carried out on H9 cell line infected by HTLV-IIIB, HTLV-MN, an African isolate RII (kindly provided by R. C. Gallo), or a French isolate Ch88 (kindly provided by D. Mathez). CEM cell line was infected by LAV-1 (kindly provided by L. Montagnier) or HTLV-RF (kindly provided by R. C. Gallo). Vero cell line was infected by VSC-25 recombinant vaccinia virus which expressed HIV gp160 (Chakrabarti *et al.*, 1986).

To determine whether antibody reactivity was directed against cell surface HIV-expressed antigenic determinants, cytofluorimetic analysis (FACScan, Becton Dickinson, Mountain View, CA) was carried out with HTLV-IIIB-infected and uninfected H9 cells and vaccinia-infected Vero cells and vaccinia gp160-infected Vero cells.

Epitope mapping

Epitope mapping was carried out by using a yeast library expressing HIV-specific peptides, as described (Madaule, Gairin & Roussier, 1991). Briefly, a random library of small fragments (50–150 bp) of HIV-1 DNA (BRU isolate) (Alizon *et al.*, 1984) was expressed in the yeast *Saccharomyces cerevisiae* with a new vector pSE-X. In this system, the recombinant peptide may undergo some post-translational modification such as glycosilation and specific proteolytic cleavage. About 10^4 yeast clones were grown on Petri dishes. Yeast colonies were then transferred onto nitrocellulose filters, lysed *in situ* and filters were analysed as Western blots.

Alternatively, epitopes were mapped with synthetic peptides. To detect epitopes recognized by sera or MoAbs synthetic peptides (kindly provided by Agence Nationale de Recherche sur le SIDA) spanning from the amino acid sequences of BRU isolate gp120: 105–117, 252–272, 302–324, 346–359, 418–434, 428–445, 432–448, 449–464, 465–479, 474–489, 487–502, 509–520, and of gp41: 583–599, 827–843, 846–860 were used in an ELISA (Neosysteme, Strasbourg, France). Each well was coated overnight in a 0·1 M sodium carbonate buffer, pH 9·5, with 10 ng of peptide to 96-well microtitre plates. Sera or MoAb were incubated for 2 h at room temperature, in PBS plus 0·2% gelatin (Fluka, Buchs, Switzerland), and then washed with PBS plus 0·2% gelatin. Bound immunoglobulins were then detected with peroxidase-conjugated goat anti-human IgG (Biosys), (1 h at room temperature). Enzyme reactions were developed using *o*-penylenediamine and read at 492 nm with a Titertek Multiskan colourimeter (Flow Laboratories).

Monoclonal immunoglobulin analysis

Isotype determination. Heavy and light chain of the MoAbs were determined by intracytoplasmic and membrane immunofluorescence on ethanol-fixed and viable cells, reacted with fluorescein-conjugated goat $F(ab')_2$ to human γ, μ, α, k, and λ (Kallestad, Austin, TX), and sheep anti-human IgG1, IgG2, IgG3, or IgG4 (Organon Teknika, Serifontaine, France) revealed by a fluorescein-conjugated rabbit anti-sheep IgG (Biosys).

Immunoglobulin production. The determination of antibody concentration in culture supernatants was performed by ELISA (Trabaud, Lery & Desgranges, 1989). Briefly, Nunc plates (Kamstrup, Denmark) were coated with goat anti-human IgG (Biosys) and incubated with culture supernatants. Bound immunoglobulins were detected with peroxidase-conjugated goat anti-human IgG (Biosys). Affinity-purified human IgGs (Hoechst-Behring, Marburg, Germany) were used to produce standard curves.

Monoclonality assessment

Genomic DNAs from antibody-secreting clones were extracted using established procedures (Maniatis, Fritsch & Sambrook 1982) and DNA from human placenta was used as control. The different DNAs were digested with *Bam*HI and *Hin*dIII enzymes (Boehringer, Mannheim, Germany). The fragments were separated on a horizontal 0·8% agarose gel which was denatured and dried. The gel was then pre-hybridized, hybridized with a labelled J_H probe and washed as described previously (Rao & Krishnamoorthy, 1987). The J_H probe (kindly provided by Dr P. Leder) consists of a 6 kb germline *Bam*HI–*Hin*dIII fragment, containing the 2·2-kb *Sau*3A fragment cloned into pBR322 overlapping the J_H2 to J_H6 region (Ravetch *et al.*, 1981). The probe was labelled by random priming with α^{32}P-dCTP (3000 Ci/mmol; Amersham, Little Chalfont, UK) to a specific activity of 10^8 ct/min per μg. The gel was exposed for 4 days to Kodak films.

Neutralization assays

Protein A-purified human MoAbs were tested in two neutralization tests. In the MT4 colourimetric assay (Robertson *et al.*, 1988), 50 μl of 2–10 TCID 50 of cell-free virus (1 TCID 50 represents 25 infectious viral particles; viral titre was determined as described by Matthews *et al.*, 1986) were mixed with 50 μl of different concentrations of MoAb against IIIB isolate (10–200 μg/ml), for 60 min at room temperature. A preparation of anti-HIV immunoglobulins from human African placenta (kindly provided by Dr G.J. Demond, Fondation Marcel Merieux, Lyon, France) was used as a positive control (ELAVIA titre, 1/1024; Western blot titre, 1/4096; neutralizing titre, 1/80).

To assess the ability of antibodies to inhibit HIV-induced cell fusion, a 50-μl aliquot of HIV-infected H9 IIIB cells ($2·5 \times 10^4$ cells) was incubated with 50 μl of the test antibodies for 60 min at 37°C and SUPT1 cells (5×10^4) were added in each well of a 96-well flat-bottomed microtitre plate and incubated at 37°C. After 5 and 18 h of incubation, the number of giant cells per well was determined by microscopic examination ($\times 40$ magnification).

Large anti-HIV MoAb production and purification

Continuous cultures were expanded in a hollow-fibre reactor (molecular cut-off, 10 kD; surface: 1·1 m^2) placed in an automated Acusyst Jr device (Endotronics, Minneapolis, MN). The recirculation flow rate of MS4 medium into the internal space of the cartridge was fixed at 250 ml/h and was increased up to 400 ml/h each time the dissolved oxygen pressure decreased below 40 mm Hg. Exchange of nutriments between the internal and the external capillary spaces was monitored by the modification of the internal pressures of the integration chamber and the expansion chamber so that 70 ml of medium were exchanged between the two spaces every 15 min. The flow rate of fresh medium introduced into the flowpath was modified daily to maintain critical levels of glucose and glutamine by means of a peristaltic pump closed to the integration chamber. The pH and dissolved oxygen pressure were automatically maintained by modifying the flow rate of air and CO_2 introduced into the gas exchange cartridge. The pH was maintained at 7·15 for 60 days and then decreased to 6·90. The flow rate of MS4 fresh medium introduced directly into the external space was identical to the harvest flow rate.

The harvested fluids were clarified by 0·22-μm Millipore filtration and loaded onto a protein A CL4B (15 ml bed volume) column (Pharmacia, Uppsala, Sweden). The anti-HIV TNBP/Tween-treated IgGs were then loaded onto a S-sepharose fast flow column (15 ml bed volume) (Pharmacia). Finally, the active fraction from the S-sepharose fast flow was gel filtered on a Superdex 20 prep grad equilibrated with PBS, pH 7·2 (300 ml bed volume). The purity was controlled by gel electrophoresis and anti-HIV activity was tested by Western blot and ELISA.

Pyrogenicity and toxicity assays

Three rabbits (NZ female hybrids) were injected intravenously with 0·5 ml/kg body weight of the purified antibody at 430 μg/ml and the rectal temperatures were then measured for 3 h. The product was declared apyrogenic if the cumulative increase of temperature on the three rabbits was not more than 1·5°C. Ten Swiss female mice were injected intravenously with 0·5 ml/20 g of the purified antibody at 430 μg/ml and examined for 7 days for survival.

RESULTS

Characteristics of the two human MoAbs

Three weeks after EBV immortalization, HIV antibodies were present in the supernatant of the two lymphoblastoid polyclonal cell lines, DZ obtained from an HIV-immunized volunteer, and C from an HIV-seropositive asymptomatic blood donor. Cell culture supernatants were screened for HIV antibodies by IIF on CEM and CEM-LAV1 infected cells and by ELISA. These two cell lines were cloned; 121 and 125 growing clones were obtained, respectively, for DZ and C: 41 clones produced anti-HIV-specific antibodies (34%) for DZ and 31 clones for C

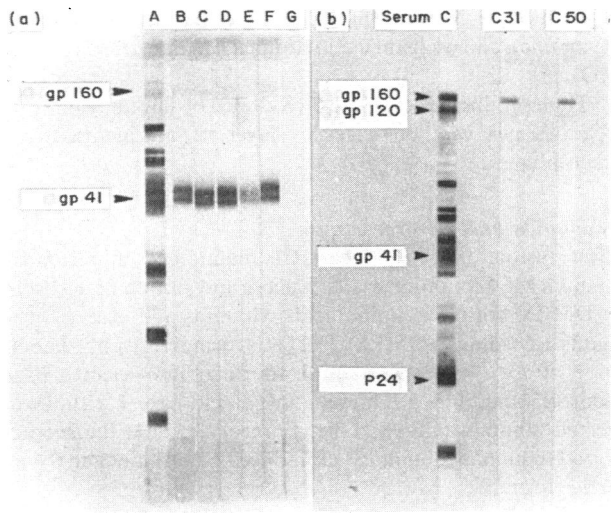

Fig. 1. Antigen specificity of the human monoclonal antibodies. Western blot analysis of supernatants from anti-HIV-1 antibody-secreting clones was performed with strips provided by Dupont (a) and Diagnostics Pasteur (b). (a) A, HIV-1 positive human serum at a 100-fold dilution; B–F, supernatants from the clones from DZ line at a two-fold dilution; G, supernatant from a clone secreting a human monoclonal antibody against hepatitis B virus, at a two-fold dilution. (b) Serum C, serum from the asymptomatic donor; C31 and C50, supernatants from the clones from C.

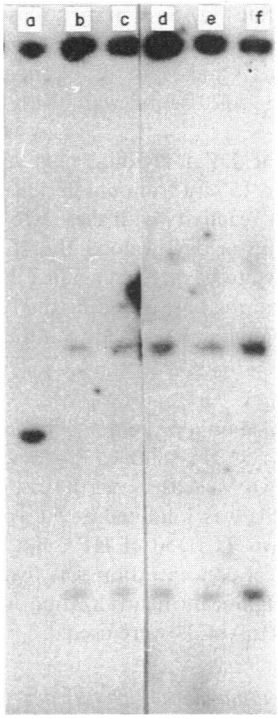

Fig. 2. Immunoglobulin gene rearrangement analysis by Southern blot of *Bam*HI and *Hin*dIII-digested DNAs hybridized with a J_H probe. Lane a, DNA from human placenta as control for the germ-line J_H fragment. Lanes b–f, DNAs from five anti-HIV secreting clones from donor DZ.

(25%). Clones secreting more than 1 μg/ml of anti-HIV immunoglobulins were selected: five clones for DZ and two clones for C.

These seven clones analyzed by Western blot showed that the five clones from DZ line were against gp160 and gp41 (Fig. 1a) and the two clones from the asymptomatic donor C were directed against gp160 and gp120 (weak) (Fig. 1b).

Monoclonality of the cell lines was assessed by the demonstration of immunoglobulin gene rearrangement with hybridization of digested cellular DNA with a J_H probe which showed that the five DZ clones were identical, since the same pattern was observed for the five clones (Fig. 2). The same was true for the two clones obtained from the asymptomatic donor, which showed a different immunoglobulin gene rearrangement pattern from that of the DZ clones (data not shown). Thus, only one antibody-producing clone of each donor was studied and designated as MoAbDZ and MoAbC31.

As seen in Table 1, the two clones produced IgG1, λ for MoAbDZ and κ for MoAbC31. Between 2 and 5 μg/ml of anti-HIV antibodies were obtained at a density of 10^6 cells/ml, for a period of 3 days for the two lines and this secretion is now stable for more than 2 years. As seen on Fig. 1, MoAbDZ recognized gp 160 and gp41 of the HTLV-IIIB isolate (Dupont kit) and MoAbC31 gp160 and gp120 of the LAV-1 isolate (Diagnostics Pasteur kit). Specific strain reactivities of the cell culture supernatants were assessed on acetone-fixed cells (Table 1); MoAbC31 was directed against all the HIV strains tested and is therefore group-specific, whereas MoAbDZ recognized only the HTLV-IIIB, HTLV-RF, and LAV-1 isolates.

Immunofluorescence analysis on viable cells was performed to examine the specificity of the two monoclonal antibodies (Fig. 3 and Table 1). MoAbC31 bound to H9 infected with HTLV-IIIB strain (Fig. 3d), whereas MoAbDZ did not (Fig. 3c) as the anti-HIV-negative serum (Fig. 3a). The fluorescence of

Table 1. Characteristics of the lymphoblastoid cell lines from the two donors

	MoAbDZ	MoAbC31
B cell origin	HIV-seronegative immunized individual (DZ)	HIV seropositive asymptomatic donor (C)
Isotype	IgG1 λ	IgG1 κ
Ig production (per 10^6 cells/3 days)	5 μg/ml	2 μg/ml
HIV specificity (WB)	gp160 and gp41	gp160 and gp120
IIF on		
H9/HTLV-IIIB	+	+
CEM/HTLV-RF	+	+
CEM/LAV-1	+	+
H9/HTLV-MN	−	+
H9/HTLV-RII	−	+
H9/HTLV-ch88	−	+
IF on viable cells*		
H9/HTLVIIIB	−	+
Vero-Vac.gp160	+	+
Epitope mapping†	827–843 846–860	Not determined‡

* Cytofluorimetric analysis.
† Epitope mapping carried out by ELISA.
‡ Not reactive with peptides described in Materials and Methods.
Ig, immunoglobulin; IIF, indirect immunofluorescence.

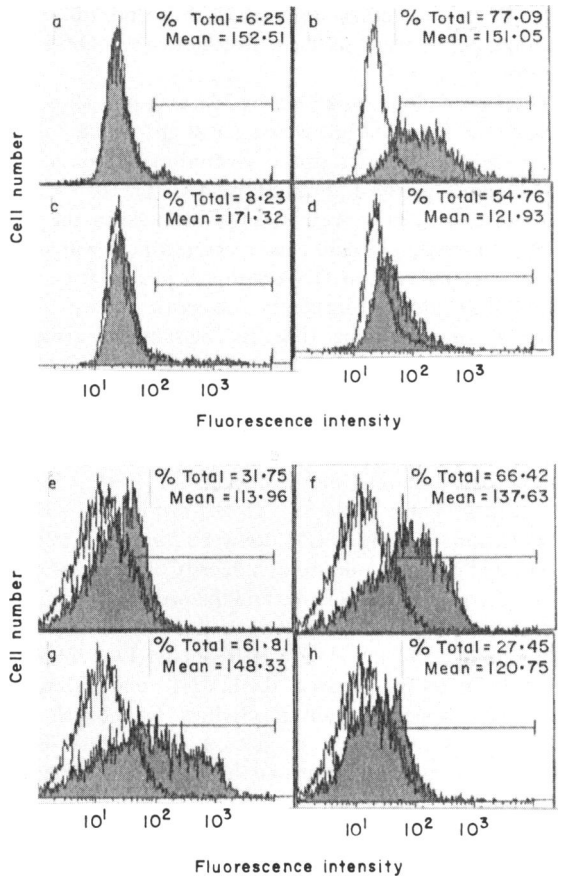

Fig. 3. Cytofluorimetric analysis. a–d, HTLV-IIIB-infected H9 cells incubated with the appropriate antibodies (solid) overlayed with HTLV-IIIB-infected H9 cells incubated with fluorescein-conjugated antibody (blank). e–h Vaccinia-gp160-infected Vero cells incubated with the appropriate antibodies (solid) overlayed with vaccinia-gp160-infected Vero cells incubated with fluorescein-conjugated antibody (blank). Appropriate antibodies: a, e, HIV-1 negative serum; b, f, HIV-1 positive serum (from the asymptomatic donor C); c, g, MoAbDZ at 50 μg/ml; d, h, MoAbC31 at 50 μg/ml.

the MoAbC31 for infected cells is not as intense as that obtained by HIV-positive serum of blood donor C (Fig. 3b). No staining was observed with the H9 non-infected cell lines (data not shown).

MoAbDZ bound to Vero cells infected by vaccinia-gp160 (Fig. 3g) like the HIV-seropositive serum (Fig. 3f), whereas MoAbC31 showed only weak fluorescence for these cells (Fig. 3h). Due to the anti-vaccine antibodies present in the human sera, it is to be noted that HIV-negative serum stained with the same intensity the Vero cells infected by vaccinia-gp160 (Fig. 3e) or vaccinia alone (data not shown).

Epitope mapping

In order to define the antigenic specificity of the MoAbs, the epitope was mapped, using a library of HIV-1 DNA fragments expressed in a yeast system. Only MoAbDZ was reacted with one yeast-recombinant clone. Sequence analysis revealed that the recognized region was located between 8272 and 8385 bp, representing amino acids 824–863 (BRU sequence). By peptide analysis (ELISA), it was shown that MoAbDZ reacted positively against the 827–843 amino acids and the 846–860 amino acids regions (Table 1). Several peptides from the gp120 envelope were tested for their reactivity with the MoAbC31 as described in Materials and Methods, but no positivity could be detected with these gp120 peptides and with HIV-1 DNA yeast library.

Serum from the DZ individual plus 25 sera from HIV-infected people and 15 sera from uninfected people were tested in ELISA for their reactivity with these two peptides (827–843 and 846–860 amino acids). Among the 26 HIV-seropositive sera, six were shown to be directed against the 827–843 and the 846–860 amino acid regions, including the DZ serum. No sera from uninfected individuals reacted with these peptides.

Neutralization assays

MoAbs were purified on a protein A column and tested in two neutralization assays. No significant syncytia inhibition was observed for the two MoAbs. Nevertheless, HIV-cytopathogenicity on MT4 cells was inhibited at 50% with 100 μg/ml of MoAbDZ with two TCID50 of HIV, but no inhibition was observed when viral concentration was five-fold higher (data not shown). No significant neutralization was observed when 10–200 μg/ml of MoAbC31 were used.

Production, purification and toxicity assays

Cloned EBV-transformed cell lines (MoAbDZ and MoAbC31) were fused to murine myeloma P3X63Age8.653. Human hybridomas obtained from these fusions were called P3XDZ and P3XC31. In order to obtain a large production of MoAbs, and to investigate problems encountered with production and purification of antibodies for treatment, an inoculum of 4×10^8 P3XC31 cells grown in MS4 medium was seeded in the external capillary space of a hollow-fibre reactor. The mean productivity of anti-HIV immunoglobulins was 6 μg/ml at the beginning of the culture, increased only after 100 days of culture. The cumulative antibody production reached 2 g after 140 days.

After the different steps of purification, it is possible to recover anti-HIV immunoglobulins from P3XC31 line with a rate of 86%. The activity of the purified MoAb, assayed by Western blotting and ELISA, was not modified when compared with culture supernatant (data not shown).

The non-pyrogenicity of the purified MoAb was demonstrated after injection into three rabbits whose cumulative increase of temperature was only 0·4°C. The 10 mice injected for the toxicity assay were alive and healthy after 7 days.

DISCUSSION

Since mice and humans can recognize antigens differently, careful interpretations must be made in regard to protective epitope identified or missed by murine MoAbs. So it is important to develop a large panel of human MoAbs.

Our study reports the production of two IgG1 human MoAbs specific for the gp160 of HIV-1. B-cell lines, derived by EBV transformation of peripheral blood were obtained from two HIV-1 seropositive individuals (DZ and C).

MoAbDZ was obtained from B lymphocytes of an individual immunized with recombinant vaccinia virus (VSC-25) expressing HIV-1 gp160 *env* protein (Chakrabarti *et al.*, 1986; Zagury *et al.*, 1987, 1988) and MoAbC31, from B lymphocytes of an asymptomatic donor.

The polyclonal lymphoblastoid cell lines derived from the two different donors and secreting anti-HIV antibodies gave rise to many anti-HIV-positive clones, but all were identical in immunoglobulin secretion for DZ (IgG1 λ) and for C (IgG1 κ). Analysis of immunoglobulin gene rearrangement demonstrated a monoclonal pattern for all the established clones secreting anti-HIV antibodies from each individual. Indeed, during the immortalization of B lymphocytes with EBV, it is usual to observe the emergence of an oligoclonal cell line with a selective growth rate (unpublished observation).

The two different clones secreted 2–5 μg/ml of IgG1 κ or λ and they have been stable with respect to growth and antibody production for over 2 years.

MoAbDZ was directed against gp160 and gp41 and reacted with fixed HTLV-IIIB-infected H9 cells, whereas no reactivity could be detected on the same viable cells. However, viable vaccinia-gp160-infected Vero cells were stained with MoAbDZ, showing that the conformation or the presentation of the gp41 in the vaccinia system is different from that in the HIV-infected T cells. The specificity of this antibody is restricted to the HTLV-IIIB and HTLV-RF isolates. MoAbDZ is directed against a region defined by the two peptides 827–843 and 846–860 amino acids; thus, the MoAbDZ epitope could be located in the region which overlaps these two peptides, or in a discontinuous region. This region is located at the gp41 carboxy terminus, which is the internal part of the protein, and is composed of an alternance of hydrophilic and hydrophobic areas (Kowalski *et al.*, 1987). Moreover, an 11% divergent nucleotide sequence is present between the IIIB and the RF strains in this region. This epitope could have been exposed during the immunization procedure as VSC-25-infected paraformaldehyde-fixed autologous cells and purified gp160 have been used for boosting (Zagury *et al.*, 1988). However, 20% of HIV-positive sera reacted with the 827–843 and 846–860 amino acid regions, indicating that the epitope can be recognized during a natural infection. As suggested by Närvänen *et al.*, (1988), an immunoreactive antigenic epitope may be hidden in intact gp41, but may be revealed and become antigenic in the course of HIV-1 infection.

MoAbC31 recognized gp160 and gp120 and reacted with fixed T cells infected by six different HIV-1 strains. Thus it binds to a conserved epitope shared by all these HIV strains. MoAbC31 is directed against an external epitope of gp120, since this monoclonal recognized epitope on viable HIV-1-infected cells. The fluorescence mean obtained with cytofluorimetric analysis for MoAbC31 was, however, much less than that for the HIV-positive serum. This may indicate that this epitope was expressed on HIV-1-infected cells but was either poorly represented on those cells or relatively accessible because of the conformation of the protein. Unfortunately, the MoAbC31 epitope was not present in all the peptides tested and not represented in the different recombinant proteins of the yeast library that we obtained. The possible site of reactivity could be defined further by exclusion analysis of the peptide tested. Indeed, considering the peptides not tested and since MoAbC31 reacted with the five HIV strains used in this study, the MoAbC31 epitope could be located in the constant region C1, C2 or C4 as it was defined by Modrow *et al.* (1987). Besides, determination of the MoAbC31 epitope by using an *E. coli* library expressing HIV-fragment proteins and some peptides localized, in the three constant regions described above is under investigation.

Fusion of HIV-infected and CD4-positive cells cannot be blocked by the two MoAbs. Nevertheless, 50% HIV-neutralizing activity was observed with 100 μg/ml of MoAbDZ and a low dose of viral particles in the MT4 assay, and 100% specific HIV-inhibition could be reached with 250 μg/ml (data not shown). Thus, a weak neutralizing activity of this MoAb was observed, which is inferior to that of the mouse MoAbs already described (Fung *et al.*, 1987; Matsushita *et al.*, 1988), which inhibited HIV infection with a concentration ranging from 0·1 to 50 μg/ml. The combination of MoAbDZ with other human MoAbs could allow a stronger neutralizing activity, as observed by Sugano *et al.* (1988). In fact, protection of mice *in vivo* against Sendai virus pneumonia was achieved by *in vitro* non-neutralizing mouse MoAbs (Mochizuki *et al.*, 1990).

The clinical use of mouse MoAbs is limited to short-term administration, since multiple injections of murine monoclonal immunoglobulins should induce a human anti-mouse immunoglobulin immune response (Shawler *et al.*, 1985). We have developed two human cell lines that synthesize human MoAbs in large quantities. As lymphoblastoid human cell lines do not grow well in synthetic medium, we fused the two human lymphoblastoid clones secreting anti-HIV antibodies with a murine myeloma. The two heteromyeloma cells secreted the same quantity of specific HIV immunoglobulins in a totally controlled synthetic medium, but their growth kinetics were increased, allowing a better antibody recuperation and concentration in a hollow-fibre reactor. In these culture conditions we got a large amount of immunoglobulins, enough to envisage therapeutic purposes; the purified immunoglobulins that we obtained were apyrogenic, and HIV specificities were preserved.

Future work will focus on well-defined neutralizing human anti-HIV antibodies cocktails; these will be useful for diagnosis and possibly treatment of HIV infection. Nevertheless, selection of HIV-1 escape mutants by neutralizing MoAbs should be considered, since this phenomenon was described *in vitro* (Reitz *et al.*, 1988; McKeating *et al.*, 1989) and *in vivo* (Nara *et al.*, 1990).

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