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REVIEW

B cell responses to HIV and the development of human monoclonal antibodies

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SUMMARY

In this review B cell responses in HIV-infected individuals are summarized together with the techniques used to date to produce human monoclonals to HIV and the properties of these antibodies. Profound disturbances in B cell responses are apparent both *in vivo* and *in vitro*. While there is evidence *in vivo* of marked polyclonal B cell activation, primary and secondary antibody responses are impaired. Similarly these cells exhibit spontaneous immunoglobulin secretion upon *in vitro* culture but do not readily respond to B cell mitogens and recall antigens including HIV. Furthermore, certain of these defects can be reproduced in normal B cells *in vitro* by incubation with HIV or HIV coded peptides. Individuals infected with HIV develop antibodies to HIV structural proteins (e.g. p17, p24, gp41 and gp120) and regulatory proteins (e.g. vif, nef, RT). Autoantibodies against a number of immunologically important molecules are also frequently observed. The anti-HIV antibodies are predominantly of the IgG1 isotype and exhibit a variety of effects on the virus *in vitro*. To date, using conventional immortalization strategies, an appreciable number of human monoclonals to HIV have been developed. These have been specific for gp41, gp120 and gag with antibodies of the former specificity predominating. The majority of these antibodies have been of the IgG1 isotype. Only a small number of the antibodies neutralize virus *in vitro* and most of these react with gp120. The neutralizing antibodies recognize conformational and carbohydrate epitopes or epitopes in amino acid positions 306–322. The predominant epitopes recognized by the anti-gp41 antibodies were in amino acid positions 579–620 and 644–662. A high percentage ($\simeq$ 25%) of these antibodies enhance viral growth *in vitro*. The problems relating to the production of human monoclonals to HIV are discussed together with strategies that could be used in the future.

INTRODUCTION

HIV is the causative organism of AIDS, a fatal disease in which the immune system breaks down, leaving the patient prey to a wide variety of viral, bacterial, fungal and parasitic infections [1,2]. Immunological abnormalities can be detected early in infection and affect to some extent all the cells of the immune system [3]. One of the main targets of HIV is the T helper lymphocyte [4–6] and there have been many studies of the consequences of this infection for T cell-related functions. However, there are also marked changes in B lymphocyte responses [7,8], although there is no evidence that HIV infects these cells.

These abnormalities have practical consequences for researchers attempting to create human MoAbs (hMoAbs) to HIV, as infected individuals constitute the principal source of immune B lymphocytes available to them. These lymphocytes are then immortalized to form cell lines that secrete specific antibody in culture. MoAbs, whether of rodent or human origin, have potential applications in the diagnosis, epidemiology and therapy of this disease and may identify viral epitopes crucial for inducing a sustained and effective immune response to the virus. This is of particular interest as, to date, there is no effective treatment for HIV infection, nor is there a suitable vaccine to prevent its transmission [9].

Here we examine in detail the cellular and humoral responses of B lymphocytes to HIV; discuss the properties and applications of hMoAbs to HIV; compare the specificities of serum antibodies with the hMoAbs presently available; and comment on the problems encountered and suggest possible future approaches.

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B CELL RESPONSES TO HIV

An essential requirement in the production of MoAbs by conventional means is that there are B lymphocytes available with the required specificity and in the most appropriate state of differentiation for them to be immortalized. In general, lymphocyte donors should be screened for the appropriate antibody, or

have been boosted recently with antigen either *in vivo* or *in vitro*, where possible. However, in the case of HIV the appropriate cells may not be available since immune abnormalities are closely associated with this infection. The major diagnostic feature of infection is a profound loss of T helper cells, coupled with a paradoxical polyclonal activation of B lymphocytes. This is accompanied by defects in cellular functions mediated by natural killer (NK) cells and monocytes, while responses to both new and recall antigens are impaired [5,10]. The precise mechanism by which all of these defects occur remains the subject of debate. While there is direct evidence that HIV infects a variety of immune cells including T helper cells and cells of the monocyte–macrophage lineage *in vivo* [11] and bone marrow progenitor cells *in vitro* [12–14], there is none to indicate that HIV infects B cells *in vivo*. Nevertheless, there is a major upset of the B cell compartment some of which cannot be related to defective T cell or monocyte function.

B cell defects in HIV-infected individuals

The predominant *in vivo* defects of B cells seen in HIV-infected individuals are outlined in Table 1. Some of these defects, such as elevated serum levels of IgD, can be detected very early in infection, before the characteristic loss of T helper cells [21]. Similarly, the performance of B cells in a variety of *in vitro* assays reflects these defects (Table 2). The activated state of these cells can be measured in their spontaneous secretion of immunoglobulin in culture, although two groups of investigators found this to be less than control cells [24,25].

The specificity of this secreted immunoglobulin has been determined in some cases. In one study [31], Western blot profiles of the supernatants sometimes corresponded with serum patterns while another [34] failed to detect specific anti-p24 activity although most patients had this serum specificity. In contrast, another group [35] could correlate spontaneous secretion of p24 IgG antibodies with their presence or absence in serum. In our experience of a small group of asymptomatics, *in vitro* immunoglobulin secretion was very variable; some individuals secreted high amounts of virus-specific immunoglobulin, particularly anti-gp120, while others secreted negligible amounts.

In one study [37], pokeweed mitogen (PWM) induced the secretion of HIV-specific antibodies from seronegative, high-risk patients; HIV infection had been confirmed by the polymerase chain reaction (PCR).

Table 1. B cell defects seen *in vivo* after infection by HIV

Effect	Ref. no.
Increased polyclonal activation	[15]
Increased serum immunoglobulin levels; IgD elevation may be an early marker	[16]
Increased levels of serum B2 microglobulin	[17]
Decreased specific antibody responses to recall antigens, e.g. polysaccharides	[7,8]
Decreased specific antibody responses to primary antigens, e.g. proteins	[7,8]
Increased proportion of immature cells in circulation	[15]
Increase in EBV-infected cells	[18,19]
Histological abnormalities of B cell zones in the germinal centres of lymph nodes	[20]
B lymphoid tumours	[20]

Table 2. Performance of B cells *in vitro* after HIV infection

Effect	Ref. no.
Reduction of proliferation in response to B cell mitogens and recall antigens	[22–24]
Reduction in development of immunoglobulin-secreting cells in response to B cell mitogens	[8,25–28]
No response to HIV or viral antigens	[29,30]
Reduced susceptibility to EBV infection *in vitro*	[19]
Spontaneous secretion of immunoglobulin in culture, some specific for HIV:	
(i) unstimulated	[15,16,19,28,31–36]
(ii) stimulated by pokeweed mitogen	[34,37]
(iii) stimulated by Epstein Barr virus	[38]

Effects of HIV on normal cells

The effects of HIV on normal cells of both immunological and non-immunological origin *in vitro* are variable. In some cases suppression similar to that seen in infected individuals is observed, whereas in others it has the opposite effect. These are summarized in Table 3.

The most interesting discrepancy is that HIV is able on the one hand to induce proliferation of and immunoglobulin secretion by normal B cells, while on the other hand it inhibits secretion of specific antibody by cells from infected individuals [30]. These effects can be mediated by a number of distinct virally coded proteins and peptides derived therefrom. For example, the carboxyl end of gp41 is implicated in the stimulatory activity [52], while two other peptides from gp41 when conjugated to bovine serum albumin (BSA) or keyhole limpet haemocyanin (KLH) inhibit proliferative responses to concanavalin A (Con A), phytohaemagglutinin (PHA) and allo-antigens [42]. These same peptides also inhibit NK cell activity [48]. This inhibition of NK cells by HIV offers a potential explanation for the paradoxical polyclonal stimulation of B cells that occurs in the face of helper T cell loss, since NK cells are postulated to be

Table 3. Effect of HIV on normal cells *in vitro*

Effect	Ref. no.
Stimulates B cells to secrete immunoglobulin	[39–41]
Induces B cells to proliferate	[39,40]
Suppresses outgrowth of bone marrow progenitor cells and impairs differentiation of T cells	[13]
Inhibits development of immunoglobulin-secreting cells in response to PWM, SAC, EBV	[40,42,43]
Inhibits proliferation in response to mitogens	[39,43,44]
Inhibits proliferation in response to recall antigens, e.g. by *tat*, gp120	[43,45–47]
Inhibits NK cell activity	[48]
Directly cytotoxic to certain cells, e.g. neuronal cells, $CD4^+$ blasts	[49–51]

PWM, pokeweed mitogen; SAC, *Staphylococcus aureus* Cowan; EBV, Epstein–Barr virus.

regulators of B cell activity [53]. Their role appears to be to suppress and lyse activated B cells and loss of such regulation could therefore result in uncontrolled B cell proliferation.

Serum antibodies to HIV

The antibody response to HIV has been studied in some detail and an overview of the specificities and functions is given in Table 4. Antibodies are developed against structural as well as regulatory viral proteins, some of which may be early markers of infection, e.g. *nef* [62], or may act as prognostic indicators, e.g. p17 [57,59], *nef* [62] or RT [59,60]. In addition, loss of p24 antibodies in the later stages of disease is associated with transition to AIDS [56,99], the higher affinity antibodies being preferentially bound into immune complexes [100]. Antibodies to gp120 tend to persist throughout infection [99,101] although their spectrum of reactivity may alter [102].

Table 4. Antibody responses to HIV infection

	Ref. no.
Specificity	
Viral antigens	
Structural proteins, e.g. p17, p24, gp120, gp41	[54–57]
Restricted heterogeneity	[58]
Regulatory proteins, e.g. *vif*, *nef*, RT	[59–64]
Autoantigens	
Anti-T cell antibodies present at all stages	[65–69]
IL-2 epitope shared with carboxyl end of gp41	[70–71]
HLA Class II epitope shared with gp41: antibodies are immunosuppressive and associated with good health	[72–74]
CH1 region of IgG shared with gp120: high levels of antibody correlate with low CD4 counts (Susal)	[75]
CD4: purified serum antibodies had no ADCC activity	[76]
Isotypes	
Most HIV reactivity in IgG1 subclass including *env*, *gag* and *3'orf*	[77–82]
IgG2: *gag*, *pol*	[78]
IgG3: *gag*, p17 associated with health	[77,78,81]
IgG4: *gag* only, especially in haemophiliacs	[77,78]
IgM,IgA: *3'orf*, *gag*, *pol*: some in absence of any HIV^+ IgG response	[77]
IgE: *gag* in haemophiliacs	[77]
Function	
Neutralization	
Strain specific and cross-neutralizing	[83–85]
Tend to correlate with antibodies to *env* and p17	[86,87]
High or increasing titres associated with stable clinical course	[88–90]
Titres do not correlate well with ELISA	[89–91]
Enhancement	
Antibodies to gp41	[92]
Enhance Fc receptor-mediated entry of HIV	[93]
Presence correlates with disease stage	[94]
ADCC	
Antibodies to gp120 and gp41 implicated	[95–97]
Broadly reactive	[96]
Lower in AIDS than asymptomatics	[98]
Some correlation with higher ELISA titres	[98]

The specificities of virus neutralizing antibodies are of major interest for passive immunotherapy and choice of potential vaccine antigens, although the titres tend to be relatively low [86,87,90]. Neutralization is largely associated with antibodies to gp120 [85–87], although there are reports of neutralizing activity in antibodies directed against gp41 and p17 [87]. This activity can be both type specific and cross-reactive, between individual isolates and across subtypes, i.e. HIV-1 and HIV-2 [83,103,104]. Higher titres of neutralizing antibodies may protect against disease progression in paediatric cases [89] and in adults [87,90], and high-affinity antibodies to a gp120 epitope appear to prevent the transmission of HIV from mother to fetus [105–107].

In many studies the sera were heat inactivated before testing but when complement-restored samples were assayed, one group of investigators detected enhancement of infection rather than neutralization [108]. The presence of these enhancing antibodies correlated with disease stage in some studies [94] but not in others [109]. Enhancing antibodies react with an immunodominant epitope on gp41 and act via the cell surface Fc receptor [92,93].

Another important biological function of antibodies is the mediation of antibody-dependent cellular cytotoxicity (ADCC). Such antibodies have been found in a high percentage of sera and have exhibited a broad range of activity against different viral strains. These antibodies have specificity for gp120 or gp41 [95–98]. The isotype distribution of antibodies to HIV is of particular interest as this will influence their effect *in vivo*. Antibodies to gp120 are almost exclusively IgG1 [78,81,82], the predominant IgG subclass, and they exhibit both neutralization and ADCC activities [79].

The fusion protein gp41 contains many interesting immunodominant epitopes including a conserved region at amino acids (aa) 587–617 which generally evokes IgG1 antibodies, although occasionally high titres of IgG2 are observed [81,110]. Banapour *et al.* [111] described a hMoAb of the IgG2 subclass that reacts with this region (see later). Sera from AIDS patients show a selective loss of antibodies to gp41 [80,110], in particular IgG2 antibodies specific for a carbohydrate determinant [112]. In contrast, the response to *gag* is much broader, IgG3 being dominant in some studies [78,81]; Khalife *et al.* [77] detected an IgG4 and IgE response to *gag* in HIV-infected haemophiliacs. Again progression of infection has been correlated with a decrease in the *gag* response by IgG subclasses other than IgG1 [80,82].

IgA responses to HIV appear to be important indicators of infection *in utero*, as the presence of immune complexes containing HIV antigen and IgA in amniotic fluid has been correlated with transmission of infection to the fetus [113]. Similarly, IgA antibodies to low molecular weight polypeptides from HIV were detected in children born to seropositive mothers [114].

Increasingly, it is being proposed that a major component in HIV pathology is autoimmunity. This is partly related to the polyclonal activation of B cells in addition to the resemblance of certain viral proteins with normal cellular components and immunologically important molecules [4].

Another area of interest is in the appearance of anti-idiotype antibodies. These have been detected prior to seroconversion by

conventional assays [115] and they appear to recognize an idiotype shared between gp120 antibodies and the CD4 molecule [115,116]. This has obvious implications for the functioning of T helper cells during the early stages of infection. Thus, although there is no direct evidence that HIV can infect B lymphocytes, the presence of viral antigens leads to major defects in their function. High serum immunoglobulin levels are indicative of constant stimulation or loss of NK control so that the circulating population consists both of maximally activated cells and immature cells. Although initially an apparently adequate response to the infection is mounted, ultimately this proves to be insufficient to contain the virus and once AIDS has developed the lymphocytes remaining are incapable of mounting any further antibody response.

MONOCLONAL ANTIBODIES

Rodent MoAbs with specificity for HIV have been developed to study several aspects of the virus [117–119]. They have specificities covering the major surface and core proteins of the virus in addition to viral regulatory proteins [reviewed in 120]. Some rodent MoAbs to the envelope proteins and to p17 can neutralize HIV [121–126], while there are MoAbs that can effect ADCC of infected cells [121]. They have also been used to characterize strain differences [118], to study certain aspects of the virus–host relationship *in vitro*, such as viral escape from neutralizing antibodies [127–129], and as the basis for diagnostic assays [130].

Therapeutic applications

One important potential use for MoAbs is as passive immunotherapy agents for prophylaxis and treatment [131]. Several viral infections are presently treated by administration of specific antibodies derived from immune blood donors, including rubella, measles, rabies and cytomegalovirus [132,133]. There are many reasons for preferring MoAbs in place of immune plasma: they provide a more uniform product, they are produced under controlled conditions and are also independent of the constant need to recruit suitable donors [reviewed in 133].

Rodent MoAbs have been used for *in vivo* applications, including reversal of transplant rejection, tumour imaging and tumour therapy [134], but a major obstacle has been the development of human anti-rodent antibodies [135]. These human antibodies can drastically reduce the half-life of rodent MoAbs *in vivo* and their appearance may lead to clinical complications. However, MoAbs should be less immunogenic [136] and will be able to recruit cellular functions such as ADCC more effectively as they possess the correct Fc portion of the antibody molecule [137].

At the present time, the range of hMoAbs is rather restricted compared with rodent MoAbs. One solution therefore has been to clone the variable region from an appropriate rodent MoAb using genetic engineering techniques and to express this with a human constant region of the required isotype to form a chimeric antibody [138–140]. If only the complementarity determining regions of rodent origin are present then essentially the molecule is a human antibody. One such mouse–human chimera has been created from a neutralizing murine MoAb [139] and passive immunization with this antibody has successfully protected a chimpanzee from HIV infection [141]. Genetic engineering can also be used to switch isotype, increase affinity or avidity, alter *in vivo* half-life and alter effector functions of MoAbs [142,143].

HIV-positive plasma has been used for passive therapy in preliminary trials and was effective at removing both viral antigen (p24) and infected cells from circulation [144–147]. However, since 90 plasma donations were required to treat 10 patients for up to 21 months in one study [146], this is not a feasible strategy on a large scale. Nevertheless, the protective effect of the mouse–human MoAb mentioned above is obviously encouraging and should provide a further stimulus to groups working in this area.

Other potential applications

In addition to their use in passive immunotherapy, hMoAbs could identify epitopes immunogenic to the human immune system and detect strain differences. These antibodies could then be used to purify the relevant antigens by immunoaffinity. Human MoAbs could also replace the human serum component in antibody-based diagnostic assays and act as defined standards for these assays to provide absolute rather than relative antibody quantification. Coupled to drugs or toxins, hMoAbs could be used to target such agents to virus-infected cells *in vitro* and *in vivo*. Finally, there is some evidence from animal immunizations that the induction of anti-idiotypic antibodies may be beneficial [148–150]. Human MoAbs would be required as immunogens for this approach. Such idiotypic specificities have already been detected in human serum [115,151].

A SURVEY OF HUMAN MONOCLONAL ANTIBODIES TO HIV

General strategies

Rodent MoAbs are generally produced by the fusion of immune spleen lymphocytes and a suitable non-secreting myeloma partner to form hybrid cell lines [152]. This strategy can also be applied to hMoAbs but there are numerous problems to be overcome [reviewed in 153,154]. In particular, there are limitations in the availability of immune lymphocytes, while the human equivalent of the rodent myeloma cell has yet to be found, although substitutes have been tried, often themselves hybrids of human and murine cells [153,154]. An alternative strategy is to use Epstein–Barr virus (EBV), a B lymphotropic herpes virus that induces polyclonal stimulation of B cells, followed in a small percentage of cells by their transformation to immortalized cell lines [155]. Many investigators now use a combination of viral transformation to increase the proportion of specific antibody-secreting cells followed by fusion with a murine or hybrid partner cell [111,156–158].

An alternative strategy is to employ molecular cloning methods to identify and isolate the sequences for specific antibody and to re-express these in a suitable cell [159,160]. Starting material may be transiently secreting EBV lines, immune B cells selected for their specificity or even the germ line sequences which are then screened for suitable reactants [161].

Strategies used to produce hMoAbs specific for HIV

Table 5 summarizes the hMoAbs presently reported with specificities for HIV antigens. They include antibodies reactive with the major structural proteins of *gag* and *env* but none reacting with the regulatory proteins. This probably reflects the major antibody specificities found in serum. Some of them

exhibit biological activity such as viral neutralization or ADCC, and others have delineated immunodominant epitopes with no biological activity, both important areas for vaccine design. Most have been produced by EBV transformation followed in some cases by hybridization and the partner cells have included a murine myeloma (P3x63AgU1), human EBV transformed lines (UC729-6; UC729HF2) and murine–human hybrids (CB-F7; SHM-D33; K6H6/B5; HMMA2.11TG/O). Only one MoAb, the mouse–human chimera referred to earlier [168] has been developed by unconventional means.

Source of immune lymphocytes

Peripheral blood lymphocytes were the main source of immune cells used for producing hMoAbs to HIV, these being the most readily available, but not necessarily the best as normally only a small percentage of antibody-secreting cells is present. How-

Table 5. Human MoAbs to HIV

Lymphocyte source*	Immortalization procedures†	Isotype	Activity‡	Ref. no
Specific for p24				
PBL	T, cyclosporin A	IgM,κ		[162]
Tonsillar B cells	T	IgG4	Cross-reactive by FA Non-n, no ADCC	[163]
PBL	T	IgGl (3 Abs) IgG2 (1 Ab) IgG3 (1 Ab)	All non-n	[164]
PBL	F, CB-F7	IgM,κ (1 Ab) IgG1,κ (2 Abs)		[165]
PBL B cells	T	IgG1,κ	Non-n	[166]
Specific for gp120				
PBL	T, cyclosporin A F, SHM-D33	IgG1,1 (2 Abs)	n, type specific	[157]
Spleen B cells	F, P3 × 63AgU1	IgG1,1	Non-n	[167]
Murine spleen	Mouse–human chimera	IgG1,κ	n, ADCC	[168]
PBL	T	IgG1(4 Abs)	n(2 Abs)	[169–171]
PBL	T	IgG1,κ	n, cross-reactive	[172]
PBL	T	(2 Abs)		[173]
PBL	T, F, HMMA2.11TG/O	IgG	n, cross-reactive	[174]
PBL	T	IgG1	Group specific	[175]
Specific for gp41				
Spleen B cells	T, F, SBC-H20	IgG2,1	Non-n, no ADCC	[111]
LN	F, UC729HF2	IgG1,κ	Non-n	[176]
PBL B cells	T after *in vivo* immunization	IgG1,1 (3 Abs) IgM,κ	n (1 Ab), IIIB, RF	[175,177]
PBL	T	IgG2 (5 Abs)	ADCC (3 Abs)	[164,178]
PBL (HIV⁻)	T after *in vitro* immunization F, K6H6/B5	IgM (3 Abs)	Non-n	[158]
PBL B cells	T	IgG1,κ IgG1,1	Non-n n	[166]
PBL B cells	T, cyclosporin A	IgG1	Non-n, no ADCC	[179]
PBL spleen	T *in vitro* immunization, F, murine myeloma	(2 Abs)	n	[173]
Multi-specific				
Spleen (HIV⁻)	F, UC729-6 after *in vitro* immunization, HIV antigen + MDP	IgM,κ/1 (3 Abs)	p24 + gp41 Non-n	[180]
LN spleen	F, P3 × 63AgU1	IgG1,κ	gp41 + 120 + 160 n, with another MoAb no ADCC	[75]
PBL	F, CB-F7	IgG1,κ	gp41 + 120	[165]

* Lymphocyte source HIV⁺ unless otherwise stated. PBL, peripheral blood lymphocytes; LN, lymph node.

† T, transformation by Epstein–Barr virus; F, fusion with cell line specified; MDP, muramyl dipeptide.

‡ n, neutralizing; non-n, non-neutralizing; ADCC, antibody-dependent complement-mediated cytotoxicity.

ever, this is different in HIV-infected individuals as their B cells are already stimulated polyclonally, although they are more refractory to EBV infection than normal (see Table 2). Our own experience confirms these observations, as B cells from HIV-infected individuals must be plated at 10 times the concentration of normal B cells to achieve 100% transformation by EBV. In addition, the high background production of anti-viral antibodies makes selection of specific EBV lines very difficult, as many apparently positive cultures prove to be negative on subsequent transfer and re-test. In one case peripheral blood lymphocytes were obtained from a volunteer deliberately immunized with a vaccinia virus recombinant expressing gp160 [175,177,181]. Interestingly, the hMoAbs produced were specific for the gp41 portion of the immunizing molecule.

Other sources of immune lymphocytes which occasionally become available include spleen, tonsil and lymph node, all of which have been used to produce HIV-specific hMoAbs. These organs normally contain a higher percentage of B cells that seem to hybridize or become transformed more readily [182,183].

In vitro boosting with antigen is often performed when producing hMoAbs as a means of increasing the percentage of specific cells prior to immortalization [183]. Again, with HIV there are problems, since viral antigens can switch off *in vitro* antibody production by lymphocytes from infected individuals [30]. Nevertheless, one group has stimulated spleen cells from an HIV-infected individual with envelope peptides and produced a mouse–human hybrid secreting antibody that inhibits syncytium formation [173].

In vitro *immunization*

Two groups have used *in vitro* immunization of normal lymphocytes as their source of immune cells and this method has a number of advantages. The antigen can be rendered uninfective or be derived from synthetic peptides, recombinant proteins, etc. Likewise, the source of B cells can be ascertained to be uninfective, and thus the work can be performed without the need for containment conditions and the product need not be screened for HIV contamination. One disadvantage is that the MoAbs produced tend to be IgM and of low affinity.

One group [158] pretreated their normal peripheral blood lymphocytes with Leu–Leu–OMe ester. This is toxic specifically for monocytes, NK cells and a subset of T suppressor cells, that have been implicated in down-regulating antigen-specific activation of B cells [184]. The other group [180] had the relative advantage of starting with normal spleen cells and stimulated them with the adjuvant peptide MDP and a denatured preparation of HIV-1 prior to fusion with a human EBV transformant.

Properties and applications of hMoAbs to HIV

Although material from HIV-infected individuals has been available since the early 1980s, it is surprising that relatively few hMoAbs have been produced, especially considering the activated state of the source lymphocytes. Undoubtedly, their resistance to EBV infection has had some influence on this. The alternative strategy of hybridization has a much lower efficiency than EBV transformation and therefore requires a larger starting sample of lymphocytes which is not always readily available.

A high percentage of the hMoAbs produced have been of the IgG isotype; this is unusual since most lines immortalized by EBV secrete IgM. Several factors may be involved in this. For example, long-term stability of the lines may be associated with IgG producers; where unfractionated peripheral blood lymphocytes have been used, then activation of HIV-infected cells may have led to further *in vitro* stimulation by viral antigens thus causing an isotype switch or the preferential selection of memory cells; as B cells from HIV-infected individuals are already hyper-activated, there may be some intrinsic difference that leads to isotype-switched cells being immortalized preferentially.

It is also interesting to note that the subclasses of hMoAbs to HIV are represented in approximately the same proportions as found in serum, with IgG1 being the most common, followed by IgG2. In addition, the isotype restrictions mentioned earlier in immune serum antibodies are also evident here, since the hMoAbs to p24 range from IgM through all the IgG subclasses, the gp120 hMoAbs are exclusively IgG1, whereas there are IgM, IgG1 and IgG2 hMoAbs to gp41.

The hMoAbs in Table 5 have been grouped according to their specificity and it will be observed that most react with the envelope glycoprotein, particularly gp41 which appears to contain several immunodominant regions. Xu *et al.* [185] investigated a panel of ten hMoAbs to gp41 and divided their reactivity into two equal groups, amino acids (aa) 590–600 (cluster I) and aa 644–663 (cluster II). This latter epitope also defined a broadly reactive target sequence for ADCC [178]. In addition, Robinson *et al.* [186] demonstrated that four of eight hMoAbs to gp41 could enhance HIV infection *in vitro* and the two domains mapped were aa 579–613 and aa 644–663. This latter sequence is conserved both among HIV-1 isolates and between HIV-2 and simian immunodeficiency virus (SIV). Two other hMoAbs reacted with immunodominant, conserved epitopes but had no neutralizing or ADCC activity [176,179]. However, one has proved to be useful in the development of an assay specific for HIV-1 [187]. It should be noted that only three of the 19 anti-gp41 antibodies described to date showed neutralizing activity. One of them was derived from an immunized volunteer [177] and neutralized several strains, and the other was tested against only one strain [166] and its epitope mapped to the amino terminal portion.

Another potential *in vivo* activity of human antibodies has been demonstrated [188] using a hMoAb to a conserved epitope of gp41. When complexed with gp160, there was enhanced presentation to a specific gp120 T cell clone presumably through FcR binding of the hMoAb to the antigen presenting cells.

In Table 6 selected references to serum antibody specificities within gp41 have been compared with those hMoAbs, and some murine MoAbs, for which precise epitopes have been delineated. This demonstrates that gp41 contains several immunodominant, conserved regions that induce antibodies with no useful function *in vivo*. In contrast to the gp41 hMoAbs, seven of the 11 hMoAbs with gp120 activity were neutralizing, either type specific or cross-reactive. Several reacted with conformational epitopes [169,172,174] and interfered with CD4 binding. These were able to neutralize divergent strains. Another broadly neutralizing hMoAb has been mapped to five discontinuous regions on gp120, some of these being close to those critical for viral entry [196]. In contrast, three neutralizing hMoAbs to the V3 loop were type-specific [157,171], although two other hMoAbs to this region could neutralize both MN and SF2 strains [197]. Finally, the mouse–human chimera prepared from a neutralizing murine MoAb was shown to express neutralizing

activity against HIV identical to the original MoAb [168]. Furthermore, the chimera was able to use the human Fc portion of the molecule to induce ADCC, a function that the murine MoAb had been unable to perform.

There are less data available on the epitopes recognized by these hMoAbs to gp120 (summarized in Table 7). The relatively large proportion of conformational, neutralizing epitopes is interesting. In addition, neutralizing mMoAbs have been raised against carbohydrate moieties [201] and now similar antibodies have been detected in normal human serum that react with gp160, gp120 and gp41 in Western blots but have no neutralizing activity [202]. Antibodies such as these could be envisaged as masking neutralization sites or enhancing infectivity through antibody or complement receptors [203].

None of the 11 *gag*-specific hMoAbs was neutralizing, although this is not surprising since this antigen is poorly expressed on the surface of infected cells and the viral core which it forms is normally hidden in the whole virus by the envelope. No data were available on their specific reactivity, but recently a panel of mMoAbs has been used to define seven immunogenic regions within the *gag* gene product [119].

Of some interest were those hMoAbs that reacted with

Table 6. Immunoreactive regions of gp41 identified by polyclonal and monoclonal antibodies

aa	Antibody	Isotype	Antibody/epitope activity	Ref. No.
466–481	Serum		Non-n, most sera positive, no disease association	[189]
467–531	hMoAb (2)	IgG1	1 n; 1 non-n	[166]
497–509	Serum		Weakly n, most sera positive	[189]
579–603	hMoAb	IgG1	Ineffective immunotoxin	[190]
579–604	hMoAb	IgG2	Poor ADCC	[178]
579–613	hMoAb (3)		Enhancing	[186]
581–597	Serum		No protection from disease progression	[191]
583–599	Serum		Immunosuppressive region, Abs associated with health	[74]
583–599	hMoAb		n	[173]
586–620	hMoAb (3)	IgG1 (2) IgG2 (1)	Enhancing	[92,192]
587–603	Serum		Immunogenic	[74]
587–617	Serum	IgG2	Major epitope	[81]
590–600	Serum		Immunogenic	[185]
590–600	hMoAb (5)		Cluster I	[185]
594–605	hMoAb	IgG2	No ADCC, non-n	[111]
598–609	Serum		Immunodominant, highly conserved epitope	[193]
598–609	Serum		Enhancing, higher levels in AIDS	[191]
599–613	Serum		Immunogenic, epitope not recognized on whole virus	[194]
603–609	Rabbit serum		n, epitope immunodominant and conserved	[193]
605–611	hMoAb	IgG1	Non-n, immunodominant epitope	[176]
632–646+677–681 +687–691	hMoAb (3)	IgM	Non-sequential determinant, non-n, no equivalent serum Ab	[158]
643–692	hMoAb	IgG1	No ADCC, non-n	[179]
644–663	Serum		Poorly immunogenic	[185]
644–663	hMoAb (5)		Cluster II	[185]
644–663	hMoAb	IgG2	ADCC	[178]
644–663	hMoAb		Enhancing	[186]
649–662	Serum		Highly conserved, poorly immunogenic	[189]
654–666	Serum		Poorly immunogenic in humans	[74]
658–682	Serum		Significant decrease in AIDS patients	[102]
729–758	Serum		Significant decrease in AIDS patients	[102]
735–752	Serum		Immunogenic	[195]
808–845+845–862	Serum		Significant decrease in AIDS patients	[102]
827–843+846–860	hMoAb	IgG1	n, IIIB and RF	[175]
848–863	Serum		Immunogenic	[74]

aa, amino acid number; n, neutralizing; non-n, non-neutralizing.

Table 7. Immunoreactive regions of gp120 identified by polyclonal and monoclonal antibodies

aa	Antibody	Isotype	Antibody activity	Ref. no.
254–274	Rabbit serum		n, does not block gp120-CD4	[198]
254–274	Human serum		Not immunogenic on gp120	[198]
297–308	Human serum		Higher frequency in ARC/AIDS but at low titre	[189]
303–338	Human serum		Reduced on progression to AIDS	[102]
306–328	hMoAbs	IgG1	n, some cross-reactive	[157–197]
316–322	hMoAb	IgG1	n, type specific (MN)	[171]
317–322	Rabbit serum		n, MN and IIIB strains	[199]
397–439	mMoAb		Blocks gp120–CD4	[200]
1–500	hMoAb	IgG1	n, blocks gp120–CD4, conformational epitope	[169]
1–500	hMoAb		n, discontinuous epitope	[196]
1–500	hMoAb	IgG	n, blocked by soluble CD4, conformational epitope	[174]
Carbohydrate sequences (3)		mMoAbs	n, cross-reactive	[201]

aa, amino acid number; n, neutralizing; mMoAb, murine MoAb.

several viral proteins in spite of extensive efforts to ensure clonality of the cell lines. This phenomenon has also been observed with mMoAbs [204]. One of these multi-reactive hMoAbs has been used in a competition ELISA containing recombinant gp160 as antigen and is capable of detecting HIV antibodies very early in infection [205].

A potential application of hMoAbs besides passive immunization involves the specific targeting of infected cells using antibodies conjugated with a toxin such as ricin A chain. This technique has been explored as a therapeutic option for various types of cancer [134] and its potential in HIV treatment has been examined *in vitro* using a hMoAb to gp41 as the targeting agent. Specific killing of HIV-infected cells could be demonstrated without affecting normal T or B cell function [206,207].

In summary, numerous hMoAbs reactive with the major structural proteins of HIV have been prepared. Some of these exhibit biological activity and have been used to define functional epitopes on the virus in addition to indicating regions that should or should not be included in a synthetic vaccine. The potential of 'humanizing' a neutralizing murine MoAb by genetic manipulation has also been demonstrated.

CURRENT PROBLEMS AND POSSIBLE SOLUTIONS

Human MoAbs

In spite of continuing efforts to improve the production of hMoAbs, there still remain problems in a number of key areas. These have been extensively reviewed [153,154] but in general revolve around the acquisition of immune lymphocytes; their efficient immortalization; the stability of cell lines; and the levels of secreted antibody. While the latter points are largely technical, the search for suitably immunized lymphocytes remains a major stumbling block, in particular for certain specificities in which highly immunized donors are unavailable. Advances in techniques for *in vitro* immunization will undoubtedly contribute to future studies, especially in the selection of lymphocyte subsets by Leu–Leu–OMe ester treatment [184] and in the use of appropriate cytokines.

The production of HIV-specific hMoAbs, however, has its own peculiar difficulties arising from both the effect that infection has on B lymphocytes *in vivo* and the immunosuppressive nature of some viral proteins. The former difficulty can only be circumvented by careful selection of donor material. Unfortunately, it is not always possible to do this in a systematic manner, because many infected individuals are not examined clinically during the early stages. It is interesting to note that the range of hMoAbs to HIV produced from seropositive donors is quite restricted, compared with that found in serum. Peripheral blood tends to be the most readily available source of lymphocytes but, in addition to the limitations mentioned earlier, this source may also be deficient in certain lymphocyte reactivities at various points throughout the disease. For example, peripheral blood lymphocytes secreting p24 antibodies *in vitro* were absent in the presence of p24 serum antibodies [34], suggesting that the secretory cells *in vivo* might be sequestered in lymphoid tissue. Other specificities such as *nef* may be more transient, and if this antibody does reappear during infection it may be associated with symptomatic disease [62].

These limitations can be overcome by using normal lymphocytes. Two groups [158,180] succeeded in producing hMoAbs to HIV by *in vitro* immunization and this route holds more promise for the future. In particular, recombinant antigens containing epitopes of interest, for example neutralization sites, could be prepared to include immuno-stimulatory sequences but omitting those that are immunosuppressive. Thus even lymphocytes from seropositive donors might be boosted *in vitro* using peptides or short recombinant sequences, as demonstrated by

Desgranges *et al.* [173], without switching off specific antibody secretion.

The future

The data presented here have clearly shown the important contribution that hMoAbs have already made to our understanding of the interactions between HIV and the immune system. They have defined epitopes involved in viral neutralization, mediation of ADCC, antigen presentation within immune complexes and the formation of anti-idiotypes. As the range of hMoAbs is extended, further epitopes will undoubtedly be uncovered. Furthermore, *in vitro* immunization of normal lymphocytes appears to be a promising route for inducing specificities not found following natural infection [158].

Further improvements in immortalization strategies would be important. Since B cells from HIV-infected donors are already stimulated it may be better to fuse them directly. PEG-mediated fusion is generally of low efficiency but electro-fusion has been shown to be highly efficient and requires smaller numbers of starting lymphocytes [208]. It is to be hoped that this technology will become more widely available.

Undoubtedly, molecular cloning techniques will be of greater importance in future, particularly since many require only a very small number of specific cells. Not only can immunoglobulin genes be cloned and expressed, but improvements in their binding capacity or other properties can be induced by site-directed mutations. In addition, bi-specific antibodies and part molecules may have potential applications [142].

CONCLUSIONS

Infection by HIV will continue to be a major source of fatal disease worldwide for many years to come, even if transmission of the virus stopped today. However, in the few years since its identification, amazing advances in molecular virology, viral isolation and cellular immunology have taken place. It is to be hoped that hMoAbs can continue to play an important role in furthering our understanding both of the interactions between virus and lymphocytes and of the immune system itself and that their potential for therapeutic applications will be realized.

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