A Mouse Monoclonal Antibody against Epstein-Barr Virus Envelope Glycoprotein 350 Prevents Infection Both In Vitro and In Vivo

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A mouse monoclonal antibody (MAb) against Epstein-Barr virus (EBV) envelope glycoprotein 350, 72A1, inhibited EBV infection of B lymphocytes in vitro. When severe combined immunodeficient mice were injected with EBV-seronegative donors’ peripheral-blood mononuclear cells and challenged with EBV, 72A1 MAb prevented development of EBV-positive tumors: none of the test mice (0/12) developed EBV-positive tumors. In contrast, 67% (8/12) of control mice developed EBV-positive tumors (P = .001). Purified 72A1 MAb was infused into 1 healthy adult and 4 EBV-seronegative children after liver transplant. No adverse reactions were seen in the adult or in 3 of the transplant recipients. The remaining patient developed a hypersensitivity reaction, thus underlining the need to humanize the MAb.

Epstein-Barr virus (EBV), a human herpesvirus, is associated with development of posttransplant lymphoproliferative disease (PTLD), particularly after primary EBV infection in previously seronegative transplant recipients. An EBV-seronegative recipient can acquire EBV from a seropositive organ donor, and primary EBV infection thus acquired is a risk factor for PTLD [1]. This transfer of donor virus via the graft has not been observed in EBV-seropositive recipients. Because latent EBV in donor B lymphocytes has to undergo lytic replication and then infect recipient B lymphocytes, it is hypothesized that preexisting neutralizing antibodies directed against envelope gp350 in EBV-seropositive patients may be important in preventing the infection of recipient cells by donor EBV isolates during the immediate posttransplant period. If this is the case, then active or passive immunization of EBV-seronegative recipients before transplant that results in production of neutralizing anti-gp350 antibodies could prevent EBV transmission via the graft and thereby reduce the risk of PTLD development.

A mouse monoclonal antibody (MAb) against EBV gp350 produced by the hybridoma cell line 72A1 has previously been shown to prevent EBV infection of B lymphocytes in vitro [2–5]. The present study was undertaken to characterize the effect of 72A1 anti-gp350 MAb in SCID mice, an in vivo preclinical model for PTLD [6, 7]. We also aimed to assess the in vivo safety of 72A1 MAb and the effects on acquisition of EBV during the immediate posttransplant period and the subsequent development of PTLD in EBV-seronegative liver transplant recipients whose organ donors were EBV seropositive.

Subjects, materials, and methods. The 72A1 cell line was obtained from Johns Hopkins University Medical School, after a transfer agreement was signed. The antibody was manufactured in accordance with European Union good manufacturing practice guidelines at the Therapeutic Antibody Centre, Oxford. A master cell bank was made and tested for potential contaminants. Cells were cultured in a hollow fiber fermentor, and 72A1 MAb was purified by chromatography on protein A, with elution at pH 3.2, followed by cation exchange on S-Sepharose at pH 4. The purified antibody was a mouse IgG1 containing κ and λ light chains in approximately equal proportions. The hybridoma had been created using P3X63Ag8, a myeloma line that secretes mouse IgG1 κ [2]. It is likely that the purified antibody was a mixture of active and inactive species, with only a minor proportion having full bivalent binding to gp350.

To determine the EBV-neutralizing titer of 72A1, 2 × 10⁶ peripheral-blood mononuclear cells (PBMCs) from an EBV-seronegative donor were incubated at 37°C for 1 h with 10-fold serial dilutions of concentrated EBV preparations and, for each virus dilution, with 10-fold serial dilutions of 72A1 MAb (starting concentration, 1 mg/mL). Cells (2 × 10⁵ per well) were cultured on microtiter plates and were observed for 4 weeks for signs of outgrowth of EBV-immortalized lymphoblastoid cell lines (LCL). Control plates were set up with PBMCs infected with EBV but without 72A1 MAb. The dilution of antibody that prevented LCL formation in >50% of wells was taken as the neutralizing titer of that particular batch of 72A1 MAb [8].
An in-house EIA was set up to capture 72A1 MAb in plasma by coating the plates with goat anti-mouse IgG (Dako) and using peroxidase-conjugated rabbit anti-mouse IgG as a detection antibody. Similarly, an in-house EIA for detection of anti-72A1 human anti-mouse antibody (HAMA) was set up using 72A1 as a capture antibody and biotin-labeled 72A1 as a detection antibody. The HAMA assay was not isotype specific and could not differentiate between IgM and IgG.

PBMCs from 4 EBV-seronegative donors were injected intraperitoneally (ip) into SCID mice. For each donor, 2 × 10^7–3 × 10^7 PBMCs were inoculated into each mouse in a group of 6 SCID mice. For each such group of 6 mice, 3 test mice were injected with 72A1 MAb ip the day before injection of PBMCs, on the day of PBMC injection, the day after (day 1), and then 3 times weekly for 3 weeks [9], whereas 3 control mice were injected according to the same schedule with PBS only. A total of 12 mice received 72A1, and 12 acted as controls. On day 1, all mice received 50 μL ip of a concentrated EBV preparation (immortalizing titer, 10^{-1}). Mice were monitored regularly and culled when they showed signs of illness or after a preset limit of 100 days. Assessment of tumor development was performed by necropsy, and EBV association was determined by routine staining for EBV nuclear antigen and small RNAs [10, 11]. Fisher’s exact test was used to analyze the in vivo data.

Infusion of 72A1 into an adult volunteer was approved by the Medicine and Clinical Oncology Research Ethics Sub-committee, Edinburgh. A pilot study on EBV-seronegative pediatric liver transplant recipients at King’s College Hospital, London, was approved by the Local Research Ethics Committee. Informed written consent was obtained from guardians.

**Results.** The purified 72A1 MAb was shown to be IgG1, as previously reported [2]. Exclusion of the presence of any human DNA and mouse xenotropic and ectotropic viruses in the hybridoma cell line was performed by the Forensic Laboratory, Lothian and Borders Police, Edinburgh (human DNA), and Q-One Biotech, Glasgow (mouse viruses). In vitro neutralizing assays showed that 72A1 MAb at a dilution of 1:100 (10 μg/mL) inhibited EBV immortalization of B lymphocytes by concentrated, 10^{-1} and 10^{-2} virus dilutions in all (100%) culture wells. Even at a dilution of 1:10,000 (100 ng/mL), 72A1 inhibited LCL outgrowth in >50% of wells when the EBV preparation was used at a 10^{-4} dilution (the virus immortalizing titer).

To test the efficacy of the 72A1 MAb preparation in preventing EBV infection in vivo, we used the development of PTLD-like lesions in SCID mice as a readout [9]. SCID mice were inoculated with PBMCs from 4 EBV-seronegative donors and then infected with EBV. On the basis of initial titration experiments to identify the 72A1 MAb half-life in SCID mice and to maintain circulating antibody levels >1 μg/mL (data not shown), test mice received 680 μg of 72A1 ip before PBMC inoculation and then 3 times weekly for 3 weeks, whereas the control mice received PBS only. After 100 days, the results showed that none (0%) of the total of 12 mice that received the antibody preparation developed ip tumors, whereas 8 (67%) out of the 12 control mice developed EBV-positive PTLD-like lesions ip (table 1). The outcome showed a statistically significant difference between the 2 groups ($P = .001$).

To assess the half-life and identify any adverse effects of 72A1 MAb on humans, an EBV-seropositive adult volunteer was injected intravenously (iv) with a single 10-mg dose of antibody. No adverse reactions occurred during or after the infusion. Peak 72A1 MAb levels persisted in serum samples (0.08–0.12 μg/mL) for 52 h after infusion, after which they gradually declined, becoming undetectable after day 43. Anti-72A1 HAMA was not detected in serum samples when tested up to 18 months after infusion. Purified 72A1 was considered safe for use, and 7 EBV-seronegative children undergoing liver transplantation from EBV-seropositive donors were enrolled (table 2). Four children (denoted “patients 1–4”) received 1 mg of 72A1 MAb/kg of body weight by iv infusions immediately before and 6 h after transplant, then every 2 days for up to 3 weeks, whereas 3 patients (“patients 5–7”) served as controls. All patients were receiving routine posttransplant immunosuppressive therapy.

Three (75%) of the 4 MAb-infused patients (patients 2–4).

**Table 1.** Effect of passive immunoprophylaxis with 72A1 monoclonal antibody (MAb) on Epstein-Barr virus (EBV)–driven lymphomagenesis in SCID mice inoculated with peripheral-blood mononuclear cells (PBMCs) from EBV-seronegative donors.

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Intraperitoneally injected PBMCs, no.</th>
<th>No. of mice with tumors/total no. of mice (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated (PBS)</td>
<td>2 × 10^7–3 × 10^7</td>
<td>8/12 (67)</td>
</tr>
<tr>
<td>Treated (72A1 MAb)</td>
<td>2 × 10^7–3 × 10^7</td>
<td>0/12 (0)</td>
</tr>
</tbody>
</table>

$^a$ $P = .001$.

**Table 2.** Demographic data, monoclonal antibody 72A1 infusion status, and outcome in the Epstein-Barr virus (EBV)–seronegative transplant recipients enrolled in the study.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age at transplant, months</th>
<th>Sex</th>
<th>Doses of 72A1</th>
<th>Time of detection after transplant, months</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11</td>
<td>M</td>
<td>11</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>16</td>
<td>M</td>
<td>6</td>
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<tr>
<td>6</td>
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<td>F</td>
<td>0</td>
<td>2.5</td>
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<tr>
<td>7</td>
<td>9</td>
<td>F</td>
<td>0</td>
<td>41</td>
</tr>
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</table>

**NOTE.** ND, not detected; VCA, viral capsid antigen; −, seronegative.
remained EBV seronegative during the 6-month follow-up period [12] (table 2). Patient 1 (1 [25%] of 4 infused) became IgG anti-viral capsid antigen (VCA) seropositive 12 weeks after transplant (9 weeks after the last MAb dose). Two (67%) of the 3 noninfused control patients (patients 5 and 6) became seropositive at 10 and 12 weeks. The remaining control patient (patient 7) seroconverted at 41 months after transplant. All seroconverted patients were seronegative for IgG to Epstein-Barr nuclear antigen (EBNA). Long-term follow-up showed that EBV DNA was detected by polymerase chain reaction 1.5–4 years after transplant in plasma from patients 1, 2, 4, 5, and 7 (performed at the Health Protection Agency Virology Laboratory, King’s College Hospital, London) (table 2), although none of these patients developed any features of EBV disease. Patient 3 remained EBV DNA, EBNA IgG, and VCA IgM and IgG seronegative for 4 years after transplant. EBV DNA was not detected in pretransplant PBMC samples from any patient when retrospective analysis was performed.

72A1 MAb was detected in serum samples from all the infused children. However, the levels were lower in the child who seroconverted at 3 months (patient 1) than in those (patients 2–4) who remained EBV seronegative at 6 months after infusions (figure 1A). All children had detectable anti-72A1 HAMA in serum samples (figure 1B). Three of the 4 infused children (patients 1–3) tolerated the infusions well, with no reactions attributable to the antibody. Patient 4 (an 18-month-old girl) had a severe reaction (peripheral cyanosis, hypotension, and vomiting) during the seventh infusion, requiring cessation of treatment. The patient also had Staphylococcus aureus septicemia. From day 7 onward, anti-72A1 HAMA levels in patient 4 serum samples were higher (maximum, 8.5 μg/mL) than those in samples from the other infused children (figure 1B). Total serum IgE levels measured in samples obtained before 72A1 infusion and samples obtained up to 18 days after 72A1 infusion from this patient were within normal range (measurements were performed at the Scottish National Blood
Transfusion Services, Edinburgh), ruling out an IgE-mediated response to HAMA. No 72A1 MAb or anti-72A1 HAMA was detected in the noninfused control patients. Four years after the transplant, all the children (infused and control) were alive with a functioning graft, and none had developed PTLD.

Discussion. EBV infects B lymphocytes via the attachment of envelope gp350 to cell surface CD21/CR2 receptors, and antibodies to gp350 are neutralizing. For this reason, research into a vaccine to prevent EBV-associated diseases has focused on gp350 to elicit a protective antibody response [13]. Thus, we have argued that it may be possible to provide short-term protection to individuals at high risk for EBV disease, such as EBV-seronegative transplant recipients, by passive infusion of anti-gp350. To this end, the present study aimed to characterize the anti-gp350 mouse MAb 72A1 and assess its potential to protect against acquiring EBV from the graft during the immediate posttransplant period, thus reducing the risk for PTLD. Initial results confirmed that, as reported elsewhere [2–5], 72A1 MAb neutralizes EBV infection of B lymphocytes in vitro, and we therefore proceeded with in vivo studies. The development of PTLD-like tumors in SCID mice after injection of PBMCs from EBV-seropositive donors can be prevented with purified immunoglobulin pooled from plasma from seropositive donors [14], suggesting that neutralizing antibody plays a role in protection against tumors. With a similar SCID mouse model, using development of PTLD-like lesions as a readout [9], we attempted to mimic the situation of EBV transfer from an EBV-seropositive donor to seronegative transplant recipients by injecting PBMCs from an EBV-seronegative donor followed by concentrated EBV. The results show that 72A1 provides complete and significant protection from EBV-driven PTLD-like lesions (table 1). Although further studies will assess whether EBV infection can be completely prevented by the antibody, the results suggest that neutralization of gp350 in vivo prevented EBV-driven lymphoproliferation.

Injection of a single large dose of 72A1 MAB into a healthy adult induced no adverse reactions and no HAMA production, and we, therefore, performed a pilot study on 4 liver transplant recipients. One of 4 infused and 2 of 3 control children seroconverted during the 6-month observation period (table 2). Although it is difficult to draw conclusions from this small group, it appears that 72A1 MAB provided short-term protection against acquiring EBV during the early posttransplant period. Detection of EBV DNA in plasma from 3 of 4 infected children (patients 1, 2, and 4) 1.5–4 years later indicated that they had acquired EBV infection without any features of EBV-related disease. Although they were on iatrogenic immunosuppression, all 4 children who received the antibody developed HAMA, and one (patient 4) developed a hypersensitivity reaction, although the actual cause of the reaction could not be identified. Thus, in vivo use of 72A1 MAB in its native form may not be safe. Further clinical studies would require either humanizing the MAB or producing a human MAB [15].

Acknowledgments

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References